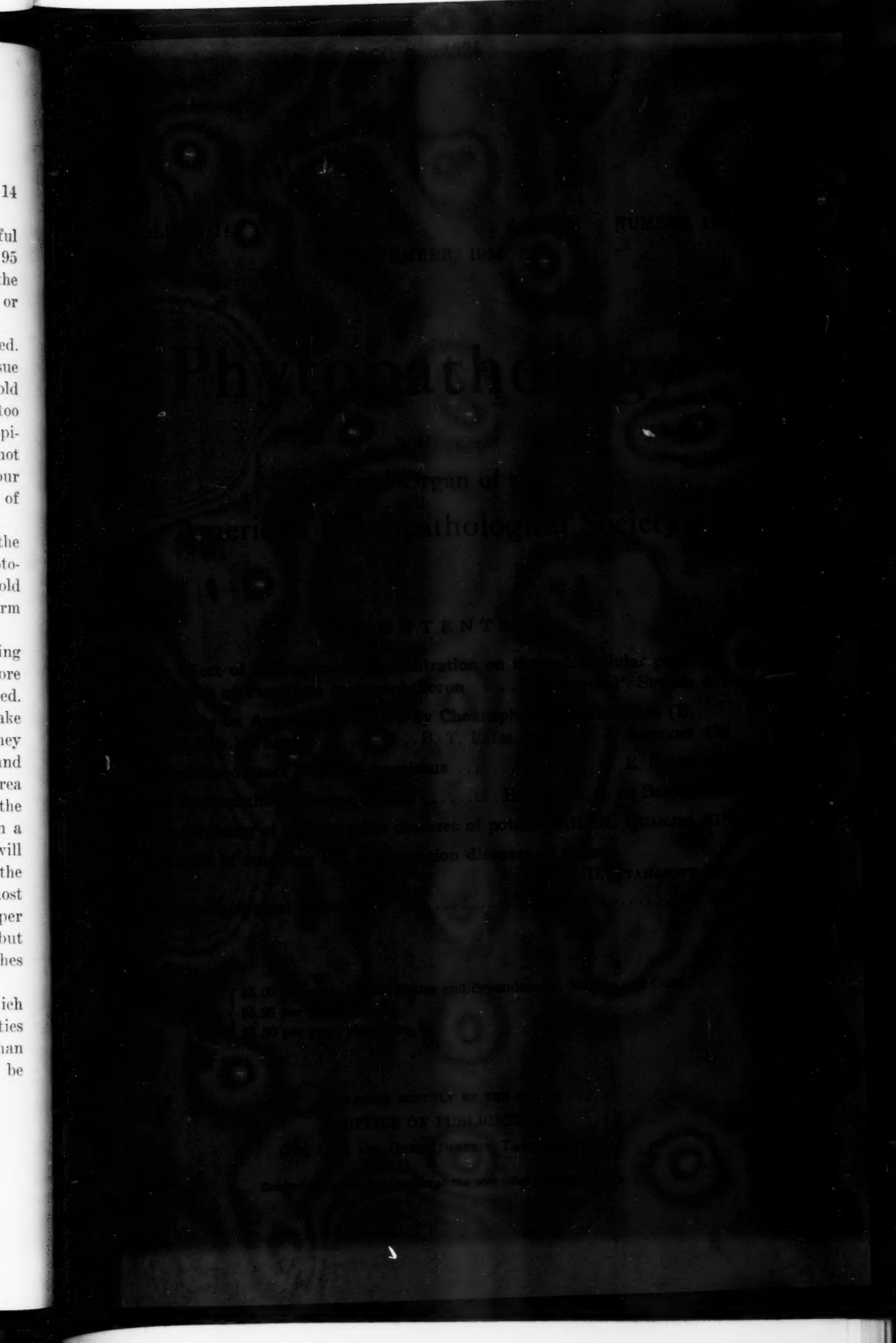

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THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE EXTRACELLULAR PECTINASE OF FUSARIUM CROMYOPHTHORON

CHRISTOS P. SIDERIS[1]

INTRODUCTION

The action of pectinase in the hydrolysis of pectin and in the maceration of vegetable tissues has been studied by many investigators. The results of these different workers indicate that distintegration of vegetable tissues by fungi and bacteria is due to an enzyme or a group of enzymes called, collectively, pectolytic enzymes. The different members of the group are recognized by their specific function: pectosinase, being the enzyme which converts pectose to pectin; pectase, the one which coagulates pectin, and finally, pectinase, that which hydrolyses pectin to reducing sugars of the pentose series, probably, to d-galactose and l-arabinose.

LITERATURE CITED

Pectinase is reported in the literature as being intracellular (that obtained from within the cell) and extracellular (that obtained from the solution surrounding the cell). Pectinase obtained from either source is shown to possess identical properties.

Jones (6) who conducted extensive researches with *Bacillus carotovorus* Jones, found that his organism secreted an enzyme which dissolves the middle lamellae considerably in advance of the invasion of the bacteria, but had no action upon the lignified or suberized walls. Brown (1) and Harter and Weimer (4) found that the macerating principle was most active in young and vigorously grown hyphae. The two latter investigators (5) showed, also, that pectinase is present in the spores of *Rhizopus tritici* Saito, and that its development is influenced considerably by the composition and the H-ion concentration of the substratum.

[1] The writer is indebted to Professor R. E. Smith and Dr. J. L. Weimer, for reading the manuscript and for helpful suggestions.

METHODS OF EXPERIMENTATION

The experiments, which follow, were planned and performed in such a way as to demonstrate the hydrolyzing principle of pectinase, obtained from substrata of a different initial H-ion concentration, on onion tissues and pure pectin.

Cultural Methods

The methods for the production of pectinase consisted in growing *Fusarium cromyophthoron* Sid. (10) in onion decoction cultures of a different initial pH, *viz.*, pH of 3.2, 4.0, 5.0, 6.0, 7.0, and 7.5, for different periods and then isolate the enzyme from the substratum. The culture solution was maintained constant in regard to the initial pH with occasional introductions of adjusting reagents, such as 0.2/N HCl and KOH.

The onion decoction was prepared as follows: 200 grams of peeled onions were cut into pieces and boiled in 500 c.c. of water which had been acidified with 25 c.c. of 5/N HCl. The entire mixture was boiled for half an hour and then filtered through cotton. The filtrate was then made up to 1,000 c.c. with a solution containing $MgSO_4$ 2.12 grams, $Ca(NO_3)_2$ 0.71 grams, KH_2PO_4 1.36 and $Fe(NO_3)_3$ 1 c.c. of a 5 per cent solution, and later adjusted to desired H-ion concentrations.

Twenty-four one-liter Erlenmeyer flasks were employed for the experiment, each one constructed after the apparatus described by Sideris (9). They were divided into four series, each one including six flasks and representing six different H-ion concentrations, *viz.*, pH of 3.2, 4.0, 5.0, 6.0, 7.0, and 7.5. All four series of cultures were inoculated at the same time, but allowed to grow for different periods: Series A, grew for seven days; B, for fifteen; C, for twenty-two, and D, for twenty-nine. The solution was filtered off from the different culture media at the end of each period and the enzyme-containing-filtrate retained while the residue consisted of the mycelial mat of the fungus thrown away.

Isolation of Pectinase

The enzyme contained in the filtrate was used in two different ways for the maceration of onion tissues: One way, was to introduce onion discs directly into the filtrate and observe the action of maceration, and the other, to isolate the enzyme from the filtrate, dissolve it in distilled water and then employ it for the maceration of onion tissues.

The isolation of the enzyme in the latter case was made in the following way: 50 c.c. of the filtrate from each culture were treated with an equal volume of 95 per cent alcohol. The mixture was let to stand for 12 hours; during this time a complete coagulation and precipitation of the enzyme was effected. The coagulated enzyme was recovered from the mixture by

filtration. Hardened filter paper[1] was used for the purpose. The enzyme was then washed from the filter paper into a beaker with distilled water heated to 40° C. The aqueous solution of the enzyme in the beaker was made up to the original volume, *viz.*, 50 c.c., by the addition of distilled water.

BEHAVIOR OF FUSARIUM CROMYOPHTHORON IN ONION DECOCTION

The different cultures were inoculated by using sporodochial macroconidia from a pure culture of *F. cromyophthoron*. Germination of the conidia took place in twenty-four hours and a fair growth of the fungus could be observed in the culture media on the fourth day.

The changes in the initial pH of each particular culture, produced by *F. cromyophthoron*, were adjusted by means of reagents, such as 0.2 normal HCl and KOH. The volume of the reagent employed for each culture represents the extent of the changes brought about by the organism. Table 1 contains the volume of the reagent added to each culture and readings of the initial and final pH.

TABLE 1.—*Volume of adjusting reagents required to neutralize the changes produced by F. cromyophthoron in the initial pH of the different cultures of series A, B, C, and D, and readings of the final pH*

Initial pH of series A, B, C, D	Series A Growth 7 days			Series B Growth 15 days			Series C Growth 22 days			Series D Growth 29 days		
	Fin. pH	Reagent H	Reagent OH	Fin. pH	Reagent H	Reagent OH	Fin. pH	Reagent H	Reagent OH	Fin. pH	Reagent H	Reagent OH
3.2	3.5	6	0	3.4	12	0	3.0	25	0	3.2	28	0
4.0	4.2	4	0	4.0	9	0	4.0	12	0	4.0	16	0
5.0	5.2	2	0	5.1	5	0	5.0	9	0	5.2	12	0
6.0	6.0	0	4	6.2	3	2	6.1	6	3	6.0	8	5
7.0	7.0	0	10	7.1	0	20	7.1	4	22	7.0	5	26
7.5	7.5	0	16	7.5	0	33	7.5	2	35	7.5	10	40

Explanation of the Results in Table 1

The results indicate that *F. cromyophthoron* is capable of effecting a marked alteration in the H-ion concentration of the external medium, the direction of the change depending upon the initial pH of the medium employed. The volume of the reagent used is an index of the amount of the changes produced by the fungus in each particular case. The seat of the

[1] The hardened filter paper was prepared by treating sheets of a 9 cm. filter paper with concentrated NaOH for two or three minutes, then washing it in distilled water until all traces of the OH-ion were removed.

direction of these changes, *e.g.*, the starting-point for an increase or decrease in the H-ion concentration of the different cultures, lies near or at pH of 5.5. The changes in the initial pH of the cultures ranging between pH of 5.5 and 3.0 were probably produced in two ways: (1) by the reaction of the CO_2 of the fungus on the water of the culture solution, resulting in the formation of CO_3-ions, and (2) by the reaction of NH_3 released from the hydrolyzed proteins of the onion decoction, on the water of the culture solution, resulting in the formation of NH_4-ions. The changes, on the other hand, in those cultures ranging in their H-ion concentration between pH of 5.5 and 7.5 were produced by a series of reverse reactions. The organism, like in the preceding case, released CO_2 in the culture solution which reacting with the water formed HCO_3-ions. Various organic acids may also be formed from the sugars of the onion decoction by the organism and thus alter the initial pH of the culture solution.

Another factor deserving consideration, in this connection, is the age of the culture. This factor is related directly, first, to the period of the utilization of the nutrient substance, and second, to the autolysis of the fungal mycelium. Both phases mentioned influence considerably the changes in the initial pH of the culture. The complete utilization of the nutrient substance may bring an ultimate stop to the metabolic activities of the organism. Autolysis being a kind of protein hydrolysis influences considerably the pH of the culture solution. The HCl added in the cultures of pH 7.0 and 7.5, of series C and D, was for the purpose of neutralizing the NH_3 released from the autolyzing fungal mycelium.

MACERATION OF VEGETABLE TISSUES

The studies on the macerating action of extracellular pectinase were conducted by placing onion discs, one cm. in diameter and three to five mm. in thickness, in Erlenmeyer flasks containing 50 c.c. of the enzymic solution. 20 c.c. of toluol were added in each flask to inhibit biological growth. The flasks were taken in an incubator at 28° C. Observations on the macerating action of the enzyme were made at 2, 3, 4, 7, 10, and 14-day-intervals. The maceration of the discs was estimated on a percentage basis; maceration of the entire disc estimated at one hundred per cent, of the one half at fifty per cent, etc.

The results on the maceration of the discs by the enzyme contained in the original filtrate are recorded in table 2 and those by the enzyme in the semi-purified condition in table 3.

Explanation of the Results in Tables 2 and 3

The results in both tables indicate quite definitely that the H-ion concentration of the substratum and the age of the culture influence consid-

TABLE 2.—*Percentage of maceration of onion discs by extracellular pectinase contained in the filtrate obtained from culture solutions of F. cromyophthoron, grown at different pH and for different periods*

Cultures		Days discs were examined	Macerating action of extracellular pectinase contained in the filtrate of cultures of different pH						
Series	Growth period		pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 7.5	Control
A	7	2	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.
		7	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.
		14	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.
B	15	3	10 pct.	10 pct.	0 pct.	20 pct.	100 pct.	0 pct.	0 pct.
		7	15 pct.	15 pct.	0 pct.	35 pct.	100 pct.	0 pct.	0 pct.
		10	20 pct.	25 pct.	0 pct.	50 pct.	100 pct.	0 pct.	0 pct.
C	22	3	20 pct.	25 pct.	50 pct.	5 pct.	3 pct.	0 pct.	0 pct.
		4	25 pct.	50 pct.	57 pct.	10 pct.	7 pct.	0 pct.	0 pct.
		7	30 pct.	100 pct.	100 pct.	15 pct.	10 pct.	0 pct.	0 pct.
D	29	3	30 pct.	75 pct.	100 pct.	5 pct.	3 pct.	35 pct.	0 pct.
		4	40 pct.	100 pct.	100 pct.	10 pct.	7 pct.	50 pct.	0 pct.
		7	50 pct.	100 pct.	100 pct.	15 pct.	10 pct.	75 pct.	0 pct.

TABLE 3.—*Percentage of maceration of onion discs by extracellular pectinase isolated from the filtrate in a semi-purified condition from culture solutions of F. cromyophthoron grown at different pH and for different periods*

Cultures		Days discs were examined	Macerating action of extracellular pectinase in a semi-purified condition from cultures of *F. cromyophthoron* of a different pH						
Series	Growth period		pH 3.2	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 7.5	Control
A	7	2	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.
		7	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.
		14	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.	0 pct.
B	15	3	0 pct.	0 pct.	0 pct.	2 pct.	100 pct.	0 pct.	0 pct
		7	0 pct.	0 pct.	0 pct.	3 pct.	100 pct.	0 pct.	0 pct.
		10	0 pct.	0 pct.	0 pct.	5 pct.	100 pct.	0 pct.	0 pct.
C	22	3	0 pct.	50 pct.	75 pct.	5 pct.	0 pct.	0 pct.	0 pct.
		4	0 pct.	75 pct.	100 pct.	10 pct.	0 pct.	0 pct.	0 pct.
		7	0 pct.	85 pct.	100 pct.	15 pct.	0 pct.	0 pct.	0 pct.
D	29	3	5 pct.	25 pct.	35 pct.	2 pct.	0 pct.	5 pct.	0 pct.
		4	10 pct.	35 pct.	50 pct.	4 pct.	0 pct.	10 pct.	0 pct.
		7	20 pct.	50 pct.	75 pct.	5 pct.	0 pct.	20 pct.	0 pct.

erably the formation and possibly the hydrolyzing action of the extracellular pectinase of *F. cromyophthoron*. It is obvious from both tables that the excretion of extracellular pectinase is favored at certain H-ion concentrations and inhibited at certain others. High concentrations of hydrogen and hydrozyl ions inhibit the excretion and possibly the formation of

extracellular pectinase, to a certain extent. The relatively diminished action of extracellular pectinase at pH of 6.0, shown practically in all series, is rather inexplicable. The writer is of the opinion that the peculiar behavior shown by the organism at this particular pH, *e.g.*, the considerably reduced activity in changing the initial pH of the culture solution, is responsible for the diminished action of the enzyme at this pH. It may be that the irritation which is produced on the fungus by slight concentrations of hydrogen and hydroxyl ions, such as at pH of 4.0, 5, and 7.2, is the cause for the excretion of pectinase at a greater rate than at pH of 6.0.

A comparative study of the results in tables 2 and 3 shows that the macerating action of the enzyme contained in the filtrate was greater than of that in the semi-purified condition. The difference in the activity is undoubtedly due to the loss of the enzyme incurred in the process of purification.

HYDROLYSIS OF PURE PECTIN

The method followed in the preceding experiment for the maceration of onion discs by extracellular pectinase in a semi-purified condition was also adapted for the hydrolysis of pure pectin. The pectin employed for the experiment was furnished by the Department of Food Products of the University of California and supposed to be free from reducing sugars. The quantity of this substance added in each flask, containing 50 c.c. of the enzymic solution, weighed 100 mgms. About 20 c.c. of toluol were added to the solution of each flask to keep it free from biological contamination and all the flasks were placed in an incubator at 28° C.

The determination of the hydrolyzed pectin of each culture was made in the following manner: The solution, containing the enzyme plus the hydrolyzed and non-hydrolyzed pectin, was treated with 10 c.c. of a 1 per cent solution of lead acetate. The Pb-ion coagulates and precipitates the enzyme and the non-hydrolyzed pectin and leaves in solution the hydrolyzed pectin. The solution was heated to 80° C. and then filtered through a hardened filter paper. The filtrate was treated with 5 c.c. of a 2 per cent solution of Na_2CO_3 to remove by precipitation the excess of the Pb-ions and then filtered for the second time. The filtrate, obtained from the second filtration, containing the hydrolyzed pectin was treated according to the method of Munson and Walker for the determination of reducing sugars.

The amount of reducing sugars, in terms of Cu_2O, obtained by the action of extracellular pectinase on pure pectin are recorded in table 4.

Explanation of the Results in Table 4

The results on the hydrolysis of pure pectin by pectinase, in table 4, do not differ essentially from those obtained in connection with the maceration of onion discs in tables 2 and 3. The weight of Cu_2O, representing

TABLE 4.—*Weight of Cu_2O in milligrams expressing the amount of reducing sugars resulted from the hydrolysis of pectin by the extracellular pectinase of F. cromyophthoron, from cultures of a different pH and age*

Cultures		Sample	Weight of Cu_2O in mgms. representing reducing sugars resulted from the hydrolysis of 10 mgms. of pectin by pectinase in 5 days at 28° C.					
			Pectinase obtained from cultures of different pH					
Series	Age		pH 3.2	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 7.5
A	7	1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
		2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
B	15	1	0.0000	0.0000	0.0000	0.0000	0.0148	0.0080
		2	0.0000	0.0040	0.0000	0.0000	0.0146	0.0072
		3	0.0000	0.0042	0.0000	0.0000	0.0142	0.0060
C	22	1	0.0040	0.0040	0.0046	0.0000	0.0100	0.0050
		2	0.0035	0.0035	0.0040	0.0060	0.0080	0.0042
		3	0.0030	0.0030	0.0036	0.0030	0.0060	0.0036
D	29	1	0.0038	0.0055	0.0146	0.0080	0.0058	0.0170
		2	0.0070	0.0128	0.0170	0.0056	0.0050	0.0149
		3	0.0060	0.0088	0.0150	0.0046	0.0030	0.0160

corresponding quantities of reducing sugars and indicating the extent of pectin hydrolysis, is analogous to the percentage of maceration expressed in the preceding experiment. The behavior of pectinase, in its relation to the pH and age of the culture, is the same in both experiments.

There is no definite knowledge on the nature of the products obtained from the hydrolysis of pectin. Considerable experimentation proved that substances such as galactose, arabinose, methyl alcohol, and acetone may all be products of pectin hydrolysis. The writer obtained negative results in an experiment in which the presence of galactose in a solution of hydrolyzed pectin was tested by the mucic acid method.

GENERAL DISCUSSION

The behavior of *F. cromyophthoron* in its relation to the excretion of extracellular pectinase varied with the age of the culture and the H-ion concentration of the substratum. The macerating action of extracellular pectinase of relatively young cultures, *viz.*, seven days old, on onion discs and pure pectin was practically nil. This behavior may be explained in two different ways: first, that pectinase does not form in relatively young cultures, and second, that if it forms it is impervious to the cell walls of very young hyphae. The excretion of pectinase, constantly found associated with older cultures, is possibly due to the increased permeability

of very old and dead cells of the fungus. It is not known whether the permeability or impermeability of the cell membrane of young hyphae is related to any extent to the saprophytic or parasitic qualifications of an organism.

The rapid release of pectinase at pH of 7.0 is possibly due to the properties of the hydroxyl ion, while the slow release at pH of 5.0 and 4.0 to the properties of the hydrogen ion. According to Osterhout (7, 8) low concentrations of hydrogen ions decrease the permeability of cell membranes, while concentrations of M .001 of NaOH increase it. The relatively small proportions of pectinase released at pH of 3.0 and 7.5 are due to the toxic properties of high concentrations of hydrogen and hydroxyl ions, which probably intercept the normal behavior of the organism. It is possible that the organism under adverse conditions may not form the same metabolic products which would otherwise under normal conditions.

The chemical reactions taking place between the pectinase and its substratum, *e.g.*, the onion discs or the pectin, are but slightly understood. This is due undoubtedly to our meagre knowledge of the molecular constitution of both the enzyme and the pectin. The chemical nature of pectin has been studied by Fellenberg (3) who found that pectin hydrolyzes in the presence of a small excess of alkali. Tutin (11), in studying the products of pectin hydrolysis obtained from the action of pectinase on pectin and from the action of alkalies found that in both cases methyl alcohol and acetone were produced. Clayston, Norris and Schryver (2) state that pectin, unless highly purified, is not a single chemical substance but a mixture of such substances. They found that by treating pectin-containing-substances with a certain concentration of NaOH, methyl alcohol and some other substances resembling hemicelluloses in their reaction, named "cytopentans" for convenience, were produced. The alkaline extract containing the methyl alcohol and cytopentans did not hold in solution any pectic substances; the latter, could be extracted from the residues with warm ammonium oxalate solution followed with HCl, the acid precipitating the pectin in the solution. Therefore, the methyl alcohol and acetone obtained in the hydrolysis of pectin by Tutin, may not result from pure pectin but from other compounds.

SUMMARY

The pectinase of *Fusarium cromyophthoron*, contained in the filtrate obtained from liquid media of different H-ion concentrations and growth periods, was used for the maceration of onion discs and the hydrolysis of pure pectin. The enzyme was used, in certain experiments, in the condition it existed in the original filtrate, and in others, in a semi-purified condition. The macerating action of the enzyme was found to decrease with purification.

In relatively young cultures the action of extracellular pectinase was found to be nil. In older cultures the action of extracellular pectinase was detected practically in every culture.

The amount of pectinase, measured by its action, secreted in the different cultures varies with the age of the culture and the H-ion concentration of the substratum. The age of the culture, affecting the longevity of the cells, influences considerably the permeability or impermeability of the cells of the fungus to pectinase. The hydrogen and hydroxyl ions, likewise, influence the rate of pectinase excretion.

Reducing sugars were obtained from the hydrolysis of pectin by pectinase. Tests for galactose gave negative results.

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A DISEASE ON AMARANTUS, CAUSED BY CHOANEPHORA CUCURBITARUM (B. & RAV.) THAXTER

B. T. Palm and S. C. J. Jochems

With Plate XXVIII

Choanephora Cucurbitarum (B. & Rav.) Thaxter has a wide distribution in the tropical and subtropical parts of the New and Old Worlds. Its occurrence in South America (Brazil) has been recorded by Möller (1921) as *Ch. americana* Möller; its distribution in North America (U. S. A.) by Wolf (1917); and in Asia (British India) by Dastur (1920). As is well known, *Ch. cucurbitacearum* not only lives saprophytically on rotting plant material, but also causes characteristic diseases on a number of plants. It attacks living flowers of *Hibiscus Rosa sinensis* L. and of other members of the same family as well as of the Cucurbitaceae family (Möller, Wolf); further it causes a not unimportant disease in the fruits of *Cucurbita pepo* (Wolf), and on species of Capsicum (Dastur).

By reason of its interest from a phytopathological point of view, we wish to put on record here a new disease caused by *Choanephora Cucurbitarum* in Sumatra, *i.e.*, on *Amarantus Blitum* L. Two other species of Choanephora have recently been reported from the Dutch East Indies by Gandrup (1923), *viz.*, *Ch. infundibulifera* (Cunn.) Sacc, and *Ch. dichotoma* n. sp.; the latter as yet without diagnosis.

Amarantus Blitum is not without importance as a substitute for *Spinacia oleracea* L. and *Portulaca oleracea* L., the culture of which apparently is difficult in tropical lowlands. This Amarantus—in the Malayan tongue "Bajam," under which name it is known also to the Europeans here—is, however, not cultivated, but simply collected from lands lying fallow, where it grows in abundance. Like most weeds in Sumatra of relatively recent introduction, it is remarkably free from parasitic fungi; till now only *Cystopus Bliti* has been observed.

When visiting a tobacco estate, situated about 50 miles above sealevel, in May, 1923, we found some specimens of "bajam" that showed an interesting disease. It is perhaps worth mentioning here that the locality was a shallow valley with a rivulet where the tobacco had died early in the season from a wilt disease (caused by *Bacterium Solanacearum* EFS) with the result that the usual growth of weeds had had an opportunity to develop luxuriantly. Half of the weed vegetation consisted of *Amarantus Blitum*; on closer inspection practically every "bajam" plant, and indeed every branch, showed the disease. Later we found the disease in the entire tobacco district of the East coast of Sumatra, on different kinds of soil,

and at different heights above sealevel. Perhaps the abnormally high humidity (for this time of the year) that prevailed in June and July this year have contributed to its wide distribution.

THE DISEASE

The disease on the "bajam" shows more or less uniformly the following symptoms. Generally the young diseased branches show a rather sharp bend, at 5 to 10 cm. from the top of the shoot (Plate XXVIII, fig. 1). From this point upwards and downwards the stem is blackened or of a dark brown color. In comparison with the undiseased portions, the stem is harder, but at the same time extremely brittle. The leaves, of the diseased parts generally hang limply, having lost their turgescence. The infected organs do not show the "wet rot" that is said to be so characteristic of the Choanephora diseases of *Cucurbita pepo* (Wolff) and Capsicum (Dastur). However, a wet rot may be found during very humid weather, but in that case the Choanephora seems always to be accompanied by an undetermined species of Fusarium; presumably the rotting is caused by the latter fungus.

In the early morning hours newly diseased organs carry a heavy crop of the characteristic conidiophores of Choanephora (Plate XXVIII, fig. 2). As already observed by Cunningham (1879) for *Ch. infundibulifera* (Cunn.) Sacc. and for our species by Möller, the conidal fructification develops only at night or during the earlier hours of the morning; this we found also to be the case in Sumatra. Sporangial fructification was only relatively seldom met with on diseased parts.

When diseased plants are collected at noon or later in the day, generally rather scanty traces of the Choanephora fructification are to be found. On the second or third day after infection has taken place there is seldom much, if anything, of the fungus to be seen, the diseased parts being practically smooth, with the exception perhaps of portions of diseased leaves.

It is said, that the infection in all diseases, caused by a Choanephora, starts in the newly opened flower. While this may be true also for the disease on Amarantus, we have not seen any evidence of such a mode of infection; our observations tend to show that the fungus may enter through other organs as well. The following infection experiments will confirm this.

As infection material we used a pure culture of Choanephora from *Amarantus Blitum*, grown on boiled rice. This culture was the third reisolation from our first culture, also on boiled rice.

Experiment I. Ten young "bajam" plants were used for inoculation with a small tuft of sterile mycelium; 5 specimens were slightly wounded by a scalpel (set A), the 5 others not injured (set B). Next day 2 speci-

mens of set A showed the typical symptoms of the disease over about 5 cm. of the stem (Plate XXVIII, fig. 3); after 2 days 2 more plants of the same set became diseased. Of set B no plants had signs of infection after 5 days.

Experiment II. Nine young "bajam" plants were infected with a suspension of conidiospores from the above mentioned pure culture, care being taken not to wound the infected spot in any way; set A (5 plants) on the upper side of the leaf, set B (4 plants) on the underside of the leaf. In set B, after 2 days, 1 plant showed signs of the disease and after 3 days 2 more plants succumbed; set A showed no results from the infection.

Experiment III. Here the underside of the leaves of 4 plants were infected, with small tufts of mycelium without wounding, the result was that after 2 days 1 plant was infected, after 3 more days all of the successful infections in these experiments resulted in the characteristic drooping of the diseased stems. These few experiments tend to show that slight wounding facilitates the infection, and further that an infection can as easily take place through the unwounded underside of a leaf. Precisely how the infecting mycelium penetrates, we did not ascertain.

In two cases this disease was found on another species of Amarantus, *A. spinosus* L. In both instances the two Amarantus species were growing intertwined, causing direct contact between diseased portions of *A. Blitum* with leaves or branches of *A. spinosus*. The symptoms resembled closely those recorded for *A. Blitum*.

Not infrequently we found black spots on the young upper leaves of *Synedrella nodiflora* (Family Compositae) associated with a bending over of the branches (Plate XXVIII, fig. 4). In one case similar symptoms were found on leaves and branches of another species of the Compositae, *Eleutheranthera ruderalis* L. The cause of the trouble was in both instances the Choanephora in question.

However, Möller has already found that in nature *Ch. Cucurbitarum* is not necessarily restricted to a living substratum; he recorded finding the fungus on rotting plant debris. Sometimes we have collected the conidial stage of a Choanephora that apparently belong to *Ch. Cucurbitarum* on dead or wilting stems of *Physalis sp.* that had been uprooted when weeding the tobacco fields for the coming rice crop. Infection experiments were, however, not undertaken.

THE CAUSATIVE FUNGUS

All authors who have studied the species of Choanephora in their natural habitat agree that only conidiophores are developed; when taken into culture, the other fructifications are formed more or less constantly. On *Amarantus Blitum*, however, in a number of instances, well developed sporangiophores have been met with on diseased parts of the plant in the

field. They seem to occur by preference on older infections intermingled with the conidial fructification. In our cultures, we invariably got both kinds of fructification, the conidial one always developing first.

These sporangiophores are, so far as we have seen, shorter and stouter than the conidiophores. They show the usual bend with its slight thickening beneath the sporangium, already noted by Möller. The darkly colored sporangia which thus are forced into a pendant position contain an average of about 100 spores. When breaking open, the sporangial wall generally splits into two halves, leaving at the base of the columella a plainly visible collar. Möller describes the sporangial wall as dark colored and finely sown with minute granulations; according to Dastur it should be smooth and colorless. For the species on Amarantus we found Möller's description confirmed. The spores from the sporangia show quite the same measurements as the conidia, *i.e.*, when compared with conidia from the same infected area. It seems worth while to mention that sporangiophores, produced in situ from parasitical mycelium in the plant regularly showed a somewhat onion-like swelling at the base, much in the same way as some Peronosporas and Sclerosporas. Möller has described a similar condition for conidiophores developed on infected parts; he found, however, that no basal swellings developed from a mycelium in artificial cultures. Wolff (*l.c.*, pl. 85, fig. 1) also gives a figure of a sporangiophore, from a pure culture, without any basal swelling.

The spores from the sporangia are provided with the characteristic hyaline appendages of about the length of the spore, are non-striated and agree completely with the description given by Wolf for *Choanephora Cucurbitarum* (B. and Rav.) Thaxter.

This is also the case with the conidia; *i.e.*, the dark violet color, the oblong form, the striation, the hyaline stump at the base as well as the size; they measure from 14 to 25 μ in length and from 8 to 12 μ in diameter.

In our cultures on boiled rice, chlamydospores of various sizes and shapes were seen; no zygospore formation has taken place as yet.

The shape of the columella is given as globular in all species. Möller (*l.c.*, p. 21) says: "Bei allen auch ist eine kuglige Columella vorhanden," Wolff and Dastur also agree on this point. Only Wolf pictures the columella as globular, the drawings published by Möller and Dastur show the columella plainly pearshaped and twice as long as broad. In the Choanephora on Amarantus the columella is rather variable in shape; from globular to pearshaped having been noted. The shape of the columella in the genus Mucor having been made the basis of classification, we have thought it useful to direct attention to this variability here. In view of the existing discrepancies between the different authors regarding size and form of the conidiophore in what is regarded as one species, a comparative

cultural study of the Choanephora from different sources should prove valuable in clearing up definitely the value to be attached to the species-characters as present accepted for the genus Choanephora.

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CHOANEPHORA CUCURBITARUM ON AMARANTUS

FIG. 1. A specimen of *Amarantus Blitum* L. attacked by *Choanephora Cucurbitarum* (B. & Rav.) Thaxter, with characteristic symptoms. (Half natural size.) FIG. 2. Conidiophores of Choanephora on diseased branch. FIG. 3. One-day-old artificial inoculation of young plant (*A. Blitum;* reduced size). FIG. 4. Diseased specimen of *Synedrella nodiflora* L. (Half natural size.)

TYLENCHUS DIPSACI KÜHN ON NARCISSUS

C. E. Scott

With Plate XXIX and Three Figures in the Text

INTRODUCTION

Narcissus bulbs have been successfully propagated on the Pacific Coast for a number of years. Commercial bulb growing in California is largely confined to the rather narrow section that comes under the modifying influence of coastal climatic conditions. The planting of narcissus bulbs has been greatly increased during the past season and the industry is receiving more attention than it has in the past. One of the troubles to be feared by the grower is the injury caused by the bulb nematode, *Tylenchus dipsaci* Kühn. This has been known as the "old disease," "eelworm disease of narcissus" and sometimes just as the "narcissus disease."

DESCRIPTION OF THE DISEASE

Under the conditions in California during the past season it has been found that the nematode injury was most apparent in the leaves and flower stems after the end of the blossoming period. At this time the severely diseased plants are much stunted, showing only half the leaf growth of healthy bulbs. These leaves are prostrate on the ground or nearly so, and in contrast to the erect habit of healthy plants except in Paper White narcissus, the thin leaves of which have a natural tendency to mat down slightly after they flower. The nematode causes a distortion of the leaves and stems. Such leaves may be twisted, with irregular margins, but the characteristic thickened specks or "spikkels," as they are called in Holland literature, are always present. To distinguish these "spikkels" from other injuries that are often found in the fields, the leaves should be drawn between the fingers. Nematode spots have the feeling of a lump that is entirely in the tissues of the leaf or stem and never on the surface. The injury may be nearer one side of the leaf but can be felt from both surfaces. The tissue of the leaf over the center of the "spikkel" may dry out, crack and become brown in color. A light yellow area surrounds the small brown spot. The slightly yellowed prostrate leaves, twisted and with irregular margins, are searched for when inspecting bulb fields for *Tylenchus dipsaci*. However, the thickened "spikkels" are the one positive evidence of the nematode disease.

When bulbs bearing strongly injured leaves are cut across, the characteristic brown ring will be revealed. Early in the season the ring will be seen as a thin brown line. This represents the layer that was infested last

season and in which the nematodes were active while in storage. After planting bulbs that are in this condition, some nematodes make their way to the new leaves of such bulbs and some to the soil. This method of spread is clearly followed in plantings of bulbs in ground which has not been previously planted to any variety of narcissus and where the bulbs have been in only during the current season. It is common to find one badly diseased plant and a half dozen plants on either side showing the leaf specks only. Figure 1 shows a group of Victoria narcissus in the same relative position as they were in the ground. The bulb in the center, bearing one small deformed leaf was responsible for spreading the disease. The nematodes have infested the two bulbs on either side. The leaves of these plants are twisted, thickened and yellowed. The leaves on the other bulbs are nearly normal in length but were found to have numerous "spikkels." Such local infestations may extend to adjacent rows at a distance of fifteen or twenty inches. Sometimes the bulb that was responsible for such an infested area will have failed to make any top growth, but will have thrown out a few roots. All degrees of infested plants will be found up to those that have made a nearly normal top growth, but showing a few "spikkels."

INJURY TO THE BULB

Upon examination of the bulb that shows severe injury in the top growth at the end of the season, one or more brown rings will be found. At first, the ring or part of the ring is but slightly discolored. As the nematodes increase by the invasion from diseased leaves and by reproduction, the brown color becomes distinctive. The diseased ring retains the normal thickness and the nematodes remain active in the tissue throughout the storage season. No evidence has been obtained to show that the nematode has the ability to pass from a diseased scale to a healthy scale adjacent to it. It is apparent that infection occurs only through the very young leaf as it pushes through the soil and the nematodes pass from that leaf to the corresponding leaf scale. However, it often happens that all the leaves of a bulb become so diseased that every scale is finally invaded.

The presence of the nematode can be demonstrated in the field by using material from a diseased bulb. A bit of diseased tissue may be put in a drop of water on a piece of glass and examined with a hand lens. An inexperienced person may readily see the active adults, and the eggs and larvae can be distinguished with but little trouble. Under favorable conditions the adults may be seen in the leaf tissue, with a hand lens, when a "spikkel" is broken, not cut across. Adults, larvae and eggs of *Tylenchus dipsaci* will be found to be numerous in the brown rings of diseased bulbs. They are present in great numbers even when only the slightest discoloration can be discerned. The nematode is not so abundant in the "spikkels" particularly

when they first appear. At that time it is common to find only a few adults in a characteristic "spikkel." Bulbs will commonly be found that have brown rings caused by insects or mites. These may be distinguished by examining a bit of the diseased tissue with the aid of the hand lens or noting the condition of the leaves with respect to "spikkels."

METHOD OF SPREAD

The nematode disease has been found in stock that has been propagated in California for twelve years, as well as in bulbs growing there in their first season from Holland, on clean land. The bulb nematode was found to have spread to an alarming extent in fields that were left without digging for two or more years. Diseased spots in these fields extend for a greater distance along the length of the rows than across the rows. Some spots included two or three rows and extended for a distance of four or five feet. One diseased area in a field of Paper White narcissus that had been in place for two years, covered five rows at the widest place and a distance of about thirty feet along two rows. This points to the hand cultivators as being an important factor in spreading the nematode in the field. It seems probable that the nematode would spread less rapidly where bulbs are grown in the Dutch bedding system. The weeding is necessarily done by hand and infested soil or diseased bulbs and leaves will not be so readily scattered. Most of the bulbs in California are grown in single rows about fifteen inches apart. This allows the use of hand operated cultivators and weed cutters.

The source of infection of a field may be judged from the conditions of the "spikkels" and appearance of the bulbs when cut. Bulbs with severely twisted leaves, and showing one or more brown rings when cut during the early part of the growing season, were diseased when planted. Such bulbs become worthless and spread nematodes to the soil and other bulbs. When healthy bulbs are planted in infested soil or near diseased bulbs, nematodes invade the young leaves and produce "spikkels" and slightly twisted leaves. Such bulbs will be free from brown rings during the early part of that growing sesaon. We have then three types of injury that will be found when diseased bulbs remain in the ground. This was seen in a field of Emperor narcissus that have been in place for twelve years. The bulbs were dead in the center of the infected area. This spot was borderd by a ring of plants which were stunted and dwarfed. Beyond this circle were the leaves of normal length but twisted and thickened. From this type the infection graded out to leaves bearing only one or two "spikkels" and with the bulbs showing no brown rings when cut (See figure 1).

CONTROL

For the control of the disease the first action that is recommended is the destruction of all diseased plants. Since the disease can be recognized best

in the growing plants, such work must be done in the field. When it occurs in a few scattered spots in the field it is practical to rogue out the plants that show nematode injury. On the other hand, the infestation may be so severe that the eradication of the diseased bulbs would not be practical. A method of treating diseased bulbs by immersion in water at a temperature

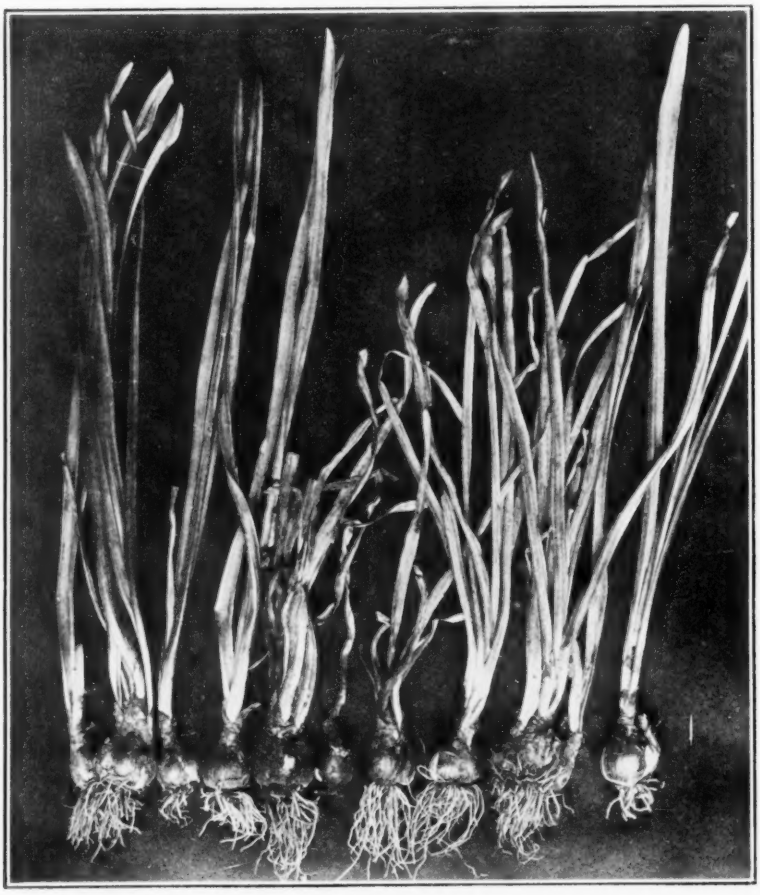


FIG. 1. Victoria narcissus photographed in same relative position in which they grew in the field. The central bulb was diseased when planted. The nematode spread to the two adjacent bulbs which produced twisted leaves. The other bulbs produced leaves with spikkels, but normal in size and with no twisting.

sufficient to kill the active nematodes and without injury to the bulbs has been developed in Holland and is used on the more valuable varieties. Van Slogteren[1] has been successful in the use of water at 110–111° Fahrenheit

[1] Van Slogteren, E. De Nematoden-Bestrijding in de Bloembollenstreek. Wageningen, 1920.

for three hours. This temperature is sufficient to kill the nematodes that have not dried out. As the bulbs stand in storage under dry atmospheric conditions some of the nematodes in the outer scales dry out and in that condition they are able to resist the temperature of 110° Fahrenheit. On this account the best time for treatment would be immediately after digging, but the greatest injury to the bulb from the hot-water treatment may result at that time. Our problem then is to determine how soon after digging can the bulb be safely given the treatment.

Preliminary experiments made during the past season indicate the possibility of developing a method that will give good control under our local climatic conditions.

METHOD OF TREATMENT

Two lots of bulbs consisting of 590 Paper White narcissus and 374 Emperor daffodils were used in a test of the hot-water treatment on August

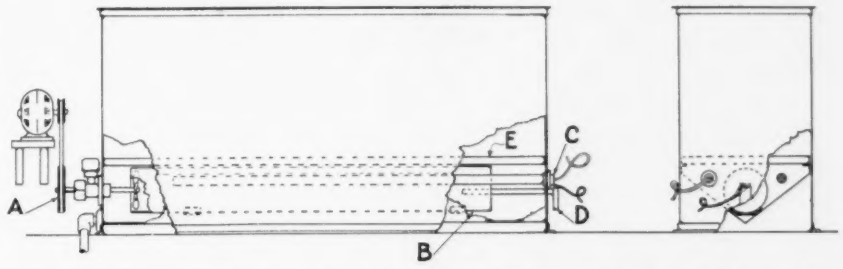


FIG. 2. Drawing of vat used for hot-water treatment of bulbs. Side view shows "A," Circulating propeller—"B," Tube to direct the flow of water and maintain circulation in all parts of the vat—"C," Electric heating elements (2) with capacity of 2.4 kilowatts at 220 volts—"D," Thermostat tube—"E," Removable screen bottom. End view shows relative positions of heating elements, thermostat tube, circulating tube and V-shaped bottom which facilitates cleaning and draining. Capacity of vat is 17 cubic feet. Designed by H. Stiner, California State Department of Agriculture.

30th. The Paper Whites had been allowed to cure in the ground and then were dug on the 18th of June. The Emperors were grown under very different climatic conditions, as they were not completely cured when removed from the ground about the first of August. Both varieties were stored under dry conditions until the time of treatment. The bulbs were sorted before treatment and all that were soft or damaged were discarded. They were further sorted according to size, the large bulbs ranging in weight from 20 to 70 grams, and the small bulbs, including splits, weighing from about 5 to 20 grams, were handled separately for one, two- or three-hour exposure to 110° Fahrenheit in warm water.

The water was heated by electric immersion units, thermostatically controlled and kept in vigorous circulation by a motor driven propeller in a vat measuring 62 inches long, 17 inches wide and 24 inches deep (Fig. 2). The water was maintained at a temperature of about 43.6°C. (110.5°F.) during the major part of the treatment, and varied between 43.2°C. (109.8°F.) and 43.8°C. (110.8°F.) Wire and wicker baskets measuring about one foot in diameter were used as containers. The bulbs were planted after treatment in unsterilized soil that was assumed to be free of *Tylenchus dipsaci*.

The green tops of the bulbs were inspected at the close of the flowering period for the presence of nematode injury. A tabulation was made which included the number of diseased plants, leaves and flower stems. These results are included in table 1. The degree of infestation was not recorded in this table.

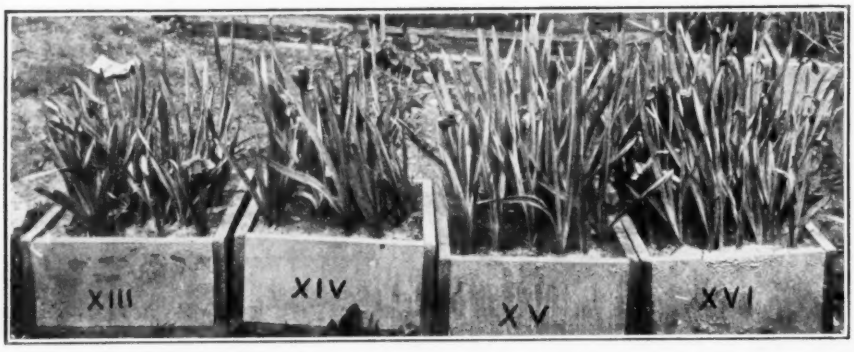


FIG. 3. Emperor narcissus photographed at the end of the blossoming period. Box XIII untreated. Box XIV treated one hour. Box XV treated two hours. Box XVI treated three hours in hot water at 110° Fahrenheit.

From these results it is apparent that a record of the severity of infestation after treatment is a better index of the effectiveness of the treament than the actual count of diseased leaves and flower stems indicate. This point is brought out both in the Paper Whites and Emperors. Box VIII (Table 1) had approximately the same number of diseased leaves as the untreated lots. But an examination of the Paper Whites toward the end of the growing season shows that the tops of the bulbs in Box XII are greener and more vigorous than the others.

The treated Emperors show a decided reduction in the number of diseased leaves. The appearance of the growing plants point decidedly toward the effectiveness of hot-water treatment. The four boxes, XIII, XIV, XV and XVI are reproduced in figure 3. These boxes were examined on the

TABLE 1.—*Results of hot water treatment on narcissus bulbs infested by Tylechus dipsaci*

Box number	Variety	Size	Treatment	Bulbs planted	Plants diseased	Number of leaves	Leaves diseased	Number of flower stems	Flower stems diseased
I	Paper White	large	untreated	50	40	522	448	36	23
II	Paper White	large	untreated	50	38	395	367	22	21
III	Paper White	large	one hour	50	37	538	321	34	16
IV	Paper White	large	one hour	49	36	456	232	24	12
V	Paper White	large	two hours	50	31	602	208	43	8
VI	Paper White	large	two hours	50	26	607	63	35	6
VII	Paper White	large	three hours	50	13	515	17	38	4
VIII	Paper White	large	three hours	50	50	525	450	31	26
IX	Paper White	splits	untreated	65	27	275	81	7	5
X	Paper White	splits	one hour	40	28	210	102	8	4
XI	Paper White	splits	two hours	40	20	238	55	16	4
XII	Paper White	splits	three hours	40	26	123	106	7	3
XIII	Emperor	large	untreated	32	26	161	115	22	6
XIV	Emperor	large	one hour	24	18	130	55	10	1
XV	Emperor	large	two hours	30	25	148	65	20	1
XVI	Emperor	large	three hours	48	35	236	75	34	0
XVII	Emperor	splits	untreated	24	16	80	31	2	1
XVIII	Emperor	splits	one hour	26	3	76	8	1	0
XIX	Emperor	splits	two hours	41	27	158	62	9	0
XX	Emperor	splits	three hours	42	35	135	95	2	0

first of June and it was found that there were green tops on only four plants in Box XIII, five in Box XIV, twelve in Box XV and twenty in Box XVI. The diseased leaves had withered and died down prematurely. The appearance of the untreated lot in Box XIII at this time are shown in plate XXIX, fig. E. Judging from the results obtained in these preliminary tests, we expect to get good control of the bulb nematode by treatment soon after digging.

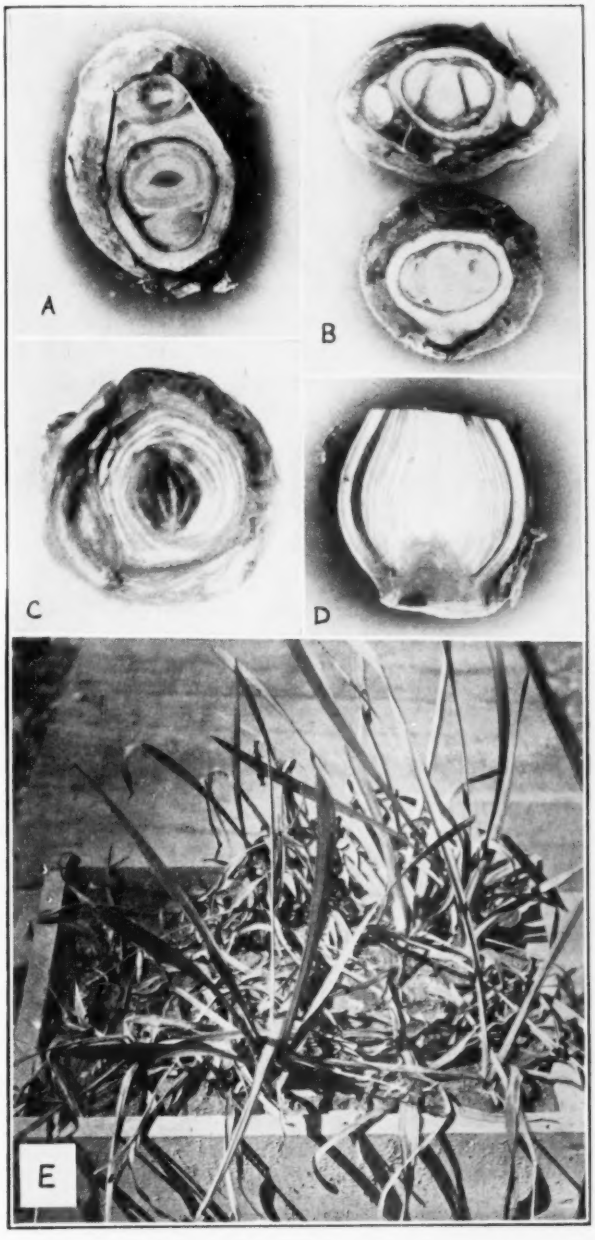
SUMMARY

Tylenchus dipsaci Kühn causes a serious disease of narcissi. Characteristic "spikkels" are formed in the leaves and flower stems which serve to identify the disease in the field. Severely infested leaves which are stunted and twisted die prematurely. The nematodes invade the bulb scales from the corresponding leaves and kill the tissue. When diseased bulbs are planted, nematodes make their way to the soil and to the young leaves as they push through the ground. Control measures that are recommended are the destruction of the diseased plants in the field and bulb treatment. Treatment of bulbs in hot-water at 110° Fahrenheit for three hours is the most promising method. Tests are being made to determine the period during which this treatment will be most effective, and will result in the minimum injury to the bulb.

CALIFORNIA STATE DEPT. AGRIC.

EXPLANATION OF PLATE XXIX

FIG. A. Paper White bulb showing narrow brown ring encircling the old bulb and two new infections. The flower stem photographed dark but was not diseased. FIG. B. Paper White bulbs that were infected when planted. FIG. C. Severely diseased bulb that was probably free from nematodes when planted. FIG. D. Vertical section of Paper White bulb showing nematode ring extending to the bulb stem. A, B, C, and D were dug and photographed after the flowering period and while the leaves were still green. FIG. E. Emperor narcissus, untreated Box XIII. This shows the premature dying of leaves badly attacked by *Tylenchus dipsaci*. Compare with figure 3, taken earlier in the season.



TYLENCHUS DIPSACI ON NARCISSUS

THE PHYTOPHTHORA DISEASE OF LILAC

HELENA L. G. DE BRUYN

WITH SIX FIGURES IN THE TEXT

INTRODUCTION

In the years 1913 and 1919 much harm was done to the lilac culture in Aalsmeer by the fungus *Phytophthora syringae* Kleb. by killing the buds and parts of the branches of the lilacs being made ready for forcing in winter. It is only in this form, occurring from January to March, that any measurable harm is done to the plants. The buds situated under the diseased part develop normally. The result will be, that if some lower branches of ordinary lilac bushes are attacked during winter, the dead ones will soon be replaced by the new developing buds in spring. For the purpose of forcing it is a quite different thing. Only a dozen branches are left on a low stem and the plants are used for forcing from November till April. The branches are long and thick and if either the buds and upper part or some other part is killed, the whole branch loses its value.

In many years not a single diseased bud was to be found, in others only a few cases were observed, while in the two years mentioned above a real epidemic occurred causing great loss to the cultivators.

The lilac trees are forced each second year, but even in the year they are not used for forcing they are pruned. If the Phytophthora should remain living in the stem, in any form whatever, the first part of the branch to be diseased should be that near the stem. The contrary is often the case, the upper parts being the first to show the disease. This and the fact that in most years diseased branches are totally absent, prove that each affected part corresponds with a new infection coming from outside.

This research was undertaken to gather some more exact knowledge about the time when infection really takes place, thus hoping to find some means of preventing new epidemics. Since the beginning of the work in the spring of 1920, circumstances have not been favorable for the attack of the branches by the fungus. Not even a dozen diseased ones could be gathered during all those years. It was therefore impossible to test the theories by the facts in nature. Still it was thought advisable to publish the results as through the experiments and observations new facts were established which might make it possible to prevent in future the outbreak of a new epidemic.

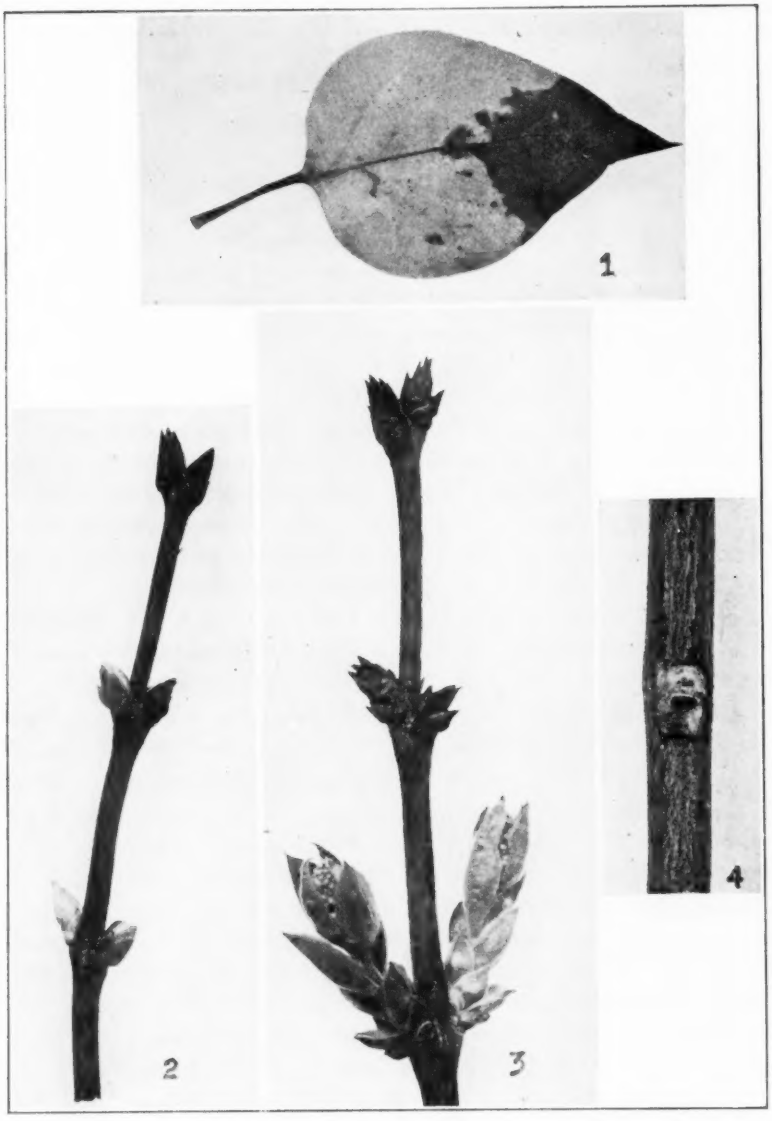


FIG. 1. Syringa leaf affected with *Phytophthora syringae*.

FIG. 2. Bud inoculation. The three upper buds are killed.

FIG. 3. The same branch as figure 2 a fortnight later. Buds of the third node developing normally. The disease makes no further advance after the buds start growth.

FIG. 4. Dark affected leaf trace; the fungus remains alive here until it can enter the cortex.

SYMPTOMS OF THE DISEASE

The occurrence of diseased lilac buds was first noticed by Klebahn (4) in 1905 in cultivations of forced lilacs near Hamburg. The fungus causing the disease was named by Klebahn (5) *Phytophthora syringae*. In this last paper he gives a full description of the outer symptoms of the diseased branches.

Buds and cortex are killed, which is to be observed by the brown coloring of both and by some shrinking of the cortex. When used for forcing, the non-development of the buds is another sign. Oospores may be present in the brown tissue of the bud scales as well as in that of the cortex and are distinctly visible after decolorizing the tissues with caustic potash.

According to Klebahn the different varieties of lilac are attacked in different ways. Of some varieties the upper parts of the branches are mostly killed, while in others the lower internodes are especially affected. In this last case the normal development of the higher buds is often prevented, especially if the killing of the cortext has gone so far that girdling of the stem takes place, preventing the normal flow of sap. In some cases two or more places of infection on the same branch can be observed, two or more diseased brown parts of the branch being separated by a green healthy piece. For the varieties of lilac used in Holland the difference of attack was described by Schoevers (10). Both authors, however, mention that the differences are not constant and that intermediate stages occur. Through the lack of occurrence of diseased branches in nature the difference of attack of the various varieties could not be studied by the writer nor its reason, which might be partly due to the different habitats of the lilac plants. The different varieties show some more or less spreading of the branches and difference in length.

It was, however, found that *Phytophthora syringae* not only attacked the buds and branches of lilacs, but that the fungus can also affect the leaves. This form of the disease does not seem to have been observed by Klebahn. On the leaves the fungus causes brown patches, which are irregular in outline, increasing gradually and occurring at any place independent of the nervature. (Fig. 1.) At the margin of the brown patch a lighter zone is observed. The color of the spots is distinctly brown, darker with the blue varieties, and always lacking the olive-green color, which is characteristic for the attack of *Heterosporium syringae* Oud.[1] (5). The affected leaves were really found in nature even at different times of the year, but only after a certain period of moist weather. *Phytophthora syringae* could be easily isolated in pure culture from them. Lilac branches inoculated in winter with Phytophthora isolated from affected leaves showed the same

[1] According to Arnaud (1) we have here to do with a Cladosporium and not with a Heterosporium.

symptoms as those inoculated with Phytophthora isolated from diseased branches.

On affected leaves, still wet with dew, sporangia were observed, proving that sporangia do occur in nature. They are very short stalked and emerge singly or in small clusters through the stomata (Fig. 5) both on the upper and lower side of the leaf; the majority, however, occurring on the lower side. They are not easily seen by the naked eye as a white haze, as is the case with *Phytophthora infestans*. In the inner part of the brown tissue of the leaves oospores were found.

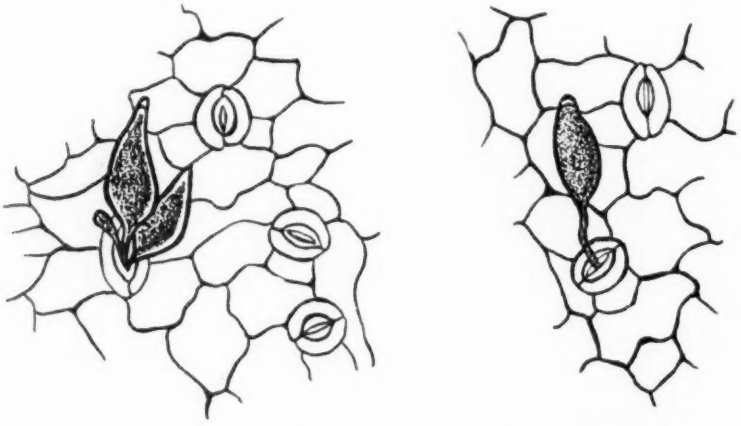


FIG. 5. Sporangia of *Phytophthora syringae* emerging from the stomata.

GEOGRAPHIC DISTRIBUTION OF THE FUNGUS

Berkeley (2) in 1881 describes, in the name of A. S. Wilson, the occurrence of large brown patches on lilac leaves, naming the fungus causing these spots, *Ovularia syringae*. He figures sporangia emerging from the stomata. Smith (11) in 1883 describes the oospores of the same fungus and A. S. Wilson (14) in 1886 the development and escape of the zoospores. G. W. Wilson (15) suggested already that *Ovularia syringae* Berk. is a synonym of *Phytophthora syringae* Kleb. Comparing the description of Berkeley of the patches on the lilac leaves and his figures with the forms found in nature, it is evident that Berkeley described the form of the disease occurring on the leaves and that *Ovularia syringae* Berk. is the same as *Phytophthora syringae* Kleb.

As already mentioned, Klebahn (4, 5) described the other form of the disease in 1905 and 1909, discovered near Hamburg, while the presence of the fungus in Holland was first made known by Schoevers (10) in 1913. In 1918 Arnaud (1) found *Phytophthora syringae* on lilacs in the garden of the Station de Pathologie végétale in Paris. Lafferty and Pethybridge

(7) in 1922 state that the rot of a couple of apples in Ireland was due to *Phytophthora syringae*.[2] They also mention having received formerly diseased lilac leaves suspected to be infested by the same fungus, but this could not be proved.

It is thus established that *Phytophthora syringae* occurs in Great Britain and Ireland, Germany, Holland, and France. It is highly probable that the fungus will also be present in other countries, the casual occurrence of leaf spots being easily overlooked.

In Holland it was proved by the presence of affected leaves that the fungus is not restricted to the lilac-growing centers for it was also detected in a garden where some isolated groups of lilacs were found.

INOCULATION EXPERIMENTS

Inoculation experiments were made to investigate the exact time of the year the fungus can live in the cortex, in which months buds will be susceptible, and if affected leaves have something to do with the attack on the stem. Therefore experiments were made by inoculating either stem, buds or leaves. With only a very few exceptions all inoculations were made on the white variety, Marie Legraye, as the brown coloring of the buds is here much more easily detected than with the blue varieties with their dark violet bud scales.

Stem Inoculations

A wound was made with a sterile scalpel in the bark, a pure culture of fungus was inserted in the wound, which was then closed and covered. Controls were treated in the same manner, using only sterile media instead of a pure culture. They all remained healthy and will therefore not be mentioned any more.

The disease developed normally when the inoculation was made in winter, but in summer it had no result. In most cases the tissue in the immediate neighborhood of the inoculation, however, was killed and turned brown, and the presence of Phytophthora mycelium was often suspected, but only a very short piece of tissue was attacked, the rest remaining healthy. This killing of the tissue may be due to some toxic substance in the medium secreted by the fungus or the fungus may be able to start some growth in the wounded cortex, which, however, soon ceases as the normal growing tissues are reached. Therefore an inoculation was only counted as successful if the cortex was brown for a distance exceeding 3 cm.

[2] It may be worth while mentioning here, that the writer with inoculations of *Phytophthora syringae*, isolated from diseased lilac branches, on apples, pears, and sour cherries still hanging on the trees got a certain percentage of rotted fruit from which the fungus could be reisolated.

In table 1 are shown the results of inoculations experiments made in the different months. It is to be seen that during the resting period the cortex is most susceptible. As soon as new growth of the lilac starts in spring, the fungus does not seem able any more to live from the tissues of the host plant. In 1923 the cambium started its function in the beginning of April. In the end of March no new tissue was observed, while on April 14th a single row of large new vessels was already formed.

The development of the fungus not only ceases as soon as the growth of the host plant starts, but the fungus seems even to die. In the diseased cortex and even in the small parts of killed tissue of the inoculations made in summer, oospores were found in quantity suggesting they might endure the unfavorable circumstances of summer; still never has any plant spread the disease in the following winter even when all the diseased branches were left on the stem, nor was it possible to reisolate the fungus in summer either from old diseased branches or from the small brown parts in the neighborhood of fresh inoculations. In winter, reisolations from diseased branches are very easily made. The few successful inoculations of July and August may be due to the fungus keeping alive till more favorable periods, perhaps in the form of oospores. The disease did not show on these branches till mid-winter. When the cambium stops growing is not known. Still its function will never be so strong in autumn as in the beginning of

TABLE 1.—*Results of stem inoculation with Phytophthora syringae*

Month and year in which inoculations were made	Number of inoculations	Number of successful inoculations. Cortex being brown for more than 3 cm.	Per cent successful
Jan., 1923	6	6	100
Feb., 1921–23	10	5	50
March, 1921–23	20	11	55
April, 1921	10	1	10
May, 1920–21	22	0	0
June, 1921–22	20	1[3]	5
July, 1920–21–22	39	2	5
Aug., 1921–22	17	2	12
Sept., 1920–21–22	21	14	66
Oct., 1921–22	22	22	100
Nov., 1920–21–22	24	24	100
Dec., 1922	12	12	100

[3] This tree was kept wet daily during 7 weeks. Of 5 inoculations only this one was successful, but the tree was in such an abnormal state, that the further course of the disease could not be followed.

spring. Therefore the fungus may perhaps keep alive during this time without yet being able to develop very much. This concerns also the inoculations made in September and October, as strong development of the fungus in the cortex only starts in December, as is demonstrated in table 2.

Table 2 shows the increase in length of diseased cortex in the different months gathered from the results of bud inoculations as well as of stem inoculations. In general the rate of growth is rather slow. Development of some importance starts only in December; in January and February maximum of growth is reached, decreasing slowly till it stops in May. In spring the part of the stem between the developing buds and a higher situated diseased piece will not be in normal condition, not getting the normal flow of sap. Therefore the fungus will in that part still be able to develop. Thus it is quite comprehensible that some growth of an already diseased branch is still possible in April while new inoculations do not succeed any more, which is demonstrated by comparing tables 1 and 2. It was often observed in spring that the disease ceased to advance as soon as the fungus reached a node with developing buds. The abnormal conditions produced by being cut off from the normal flow of sap may also be the reason for the difference in growth in both directions, the development of the disease in the top part decreasing less in spring.

The conclusion to be drawn from both tables is that *Phytophthora syringae* can invade the living tissues of the cortex of the lilac stem from December till April.

TABLE 2.—*Increase in length of diseased cortex*

Month and year in which observations were made	Increase in length of diseased cortex in direction of top		Increase in length of diseased cortex in direction of stem	
	Number of observations	Average increase in length	Number of observations	Average increase in length
Oct., 1920	3	0.0 cm.	6	0.1 cm.
Nov., 1920–22	15	0.1 cm.	18	0.2 cm.
Dec., 1920–22	21	1.9 cm.	21	1.2 cm.
Jan., 1921–23	11	1.9 cm.	38	3.0 cm.
Feb., 1921–23	8	3.0 cm.	82	3.1 cm.
March, 1921–22–23	11	2.9 cm.	131	2.3 cm.
April, 1921–22–23	18	1.9 cm.	208	1.0 cm.
May, 1920–21–23	10	1.0 cm.	27	0.7 cm.
June, 1920	7	0.5 cm.	7	0.1 cm.
July, 1922	5	0.0 cm.	5	0.0 cm.

Bud Inoculations

A series of bud inoculations was started in 1922, as preliminary experiments showed that, even as the cortex, the buds are not always susceptible to infection. Each fortnight a number of inoculations was made with the same strains of the fungus cultivated as much alike as possible. A piece of pure culture was brought between the bud scales. The 4 upper buds of 10 to 12 branches were inoculated at the same time. Thus the number of inoculations could be rather large, the drawback, however, was that the buds of one branch influenced each other to some extent. As, however, all branches were in the same condition, the results are quite comparable, only the percentage, especially of the very first ones, is perhaps a little too high. At the same time three branches were used as controls, a piece of sterile medium being brought between the bud scales. All controls remained healthy.

On many buds, brown spots were formed soon after the inoculation was made. These spots, however, did not always increase in size and the buds developed quite normally in spring. This spotting must be due to the substances formed by the fungus and present in the piece of medium used. To prove this, some cultures were heated for half an hour at 52° C. by which proceeding the fungus was killed. If a piece of this medium was used for inoculation, the majority of the buds showed the same brown spots and developed also quite normally in spring.

If the inoculation was, however, successful, the brown spots increased in size, till the whole bud was killed and after some time the cortex under the bud was attacked as well (Figs. 2, 3). None of the buds were quite brown before December. Inoculations made in September, October, and November were in December at the same stage; those of November even being distinctly in advance of those made earlier. Inoculations made after November showed the first brown bud after about five weeks. Therefore, it is highly probable that real susceptibility of the buds dates from the end of October, the successful inoculations of September and beginning of October being due to the fungus keeping alive between the bud scales.

Table 3 gives the results of the bud inoculations, those of the preliminary experiments in 1921 are added as well. Figure 6 shows a graph of both. After the maximum of November and December the susceptibility decreases gradually till it reaches a minimum in January, increasing again afterwards. This is followed by a new decrease till all susceptibility ceases with the development of the lilac buds. Both lines of the two years correspond rather well. The diminishing of susceptibility in January is not due to weather conditions, as may be thought, as in 1922–23 the temperature was rather regular during the whole winter, the first cold period occurring

TABLE 3.—*Bud inoculations with Phytophthora syringae*

Date on which inoculations were made	1922–1923			1920–1921		
	Number of buds inoculated	Diseased buds	Per cent diseased	Number of buds inoculated	Diseased buds	Per cent diseased
8 Sept.	40	0	0			
22 Sept.	40	20	50			
6 Oct.	52	30	57			
20 Oct.	48	44	91			
3 Nov.	48	48	100			
17 Nov.	48	41	85			
1 Dec.	48	48	100			
15 Dec.	48	36	75			
29 Dec.	48	34	71			
30 Dec.				16	14	87
12 Jan.	48	15	31			
14 Jan.				43	28	65
26 Jan.	48	33	69			
4 Feb.				20	17	85
9 Feb.	48	29	60			
18 Feb.				20	10	50
23 Feb.	48	24	50			
8 March				18	3	16
9 March	48	4	8			
18 March				22	2	9

only in February. If a graph is made of the number of diseased buds 8 weeks after inoculation or of the augmentation of diseased buds recorded each week, the same kind of curve is the result. This indicates that it is the real internal condition of the buds which influences the development of the fungus and not external circumstances.

The conclusion to be drawn from the bud inoculations is that lilac buds are more or less susceptible to infection of *Phytophthora syringae* from about the end of October till the end of February. This period corresponds with the time found by Versluys (12) for the rest period of the buds of Marie Legraye.

The decrease of susceptibility in January may be attributed to some other cause. Larkum (9) states that lilac buds contain their maximum of starch in November, the quantity decreases till a minimum is reached in the midst of January. In the beginning of February the quantity of starch augments again till a new maximum is reached with the beginning of the development of the buds. This change in food corresponds with the greater or lesser susceptibility of the buds during winter months. The entire

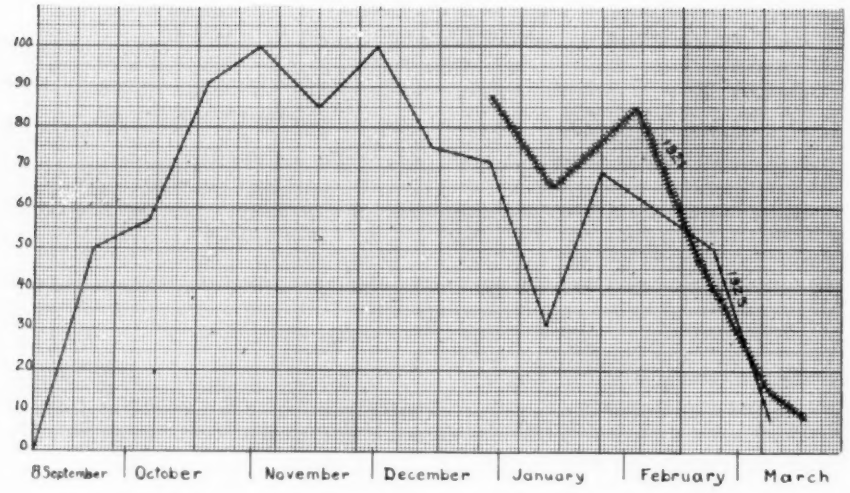


FIG. 6. Results with bud inoculations at different seasons of the year.

ceasing of the development of the disease, as soon as growth of the host tissue starts, cannot, however, be attributed to this change of food as a new maximum of starch is recorded in March. This non-growing of the fungus in developing tissues is thus found in the buds as well as in the cortex, the earlier growth of the buds causing the spreading of the disease in the buds to cease at an earlier date.

According to the theories of some authors, Lakon (8), Klebs (6), the accumulation of assimilation products by inactivating enzymes is the cause of the rest period of plants, while growth is due to the enzymes regaining gradually their activity. Weber (13) thinks that the increased activity of enzymes in developing tissues is not the cause of growth but a secondary factor. However this may be, increased activity of enzymes in growing parts is also accepted by him. This increase may explain the fact that *Phytophthora syringae* cannot live in the growing tissues of the host plant. It must be supposed, then, that the enzymes present in the growing parts are reversed to the development of the fungus and that their partial or entire inactivity during the rest period only enables the parasite to develop.

Leaf Inoculations

For the inoculations of the leaf not only the variety Marie Legraye was used, but also different seedlings. The best method proved to be to put a piece of pure culture on the leaf, covering it with some moist cotton wool and keeping this together by rolling and binding the whole leaf. In this manner some 90 inoculations were made in August, September, and October

of different years, resulting in 89 diseased leaves. Controls were treated alike, a piece of sterile medium being used instead of the pure culture. They all kept healthy. Most of the affected leaves fell prematurely. This premature dropping of the affected organ is a feature mentioned in many Phytophthora diseases. Of the 89 diseased leaves only 4 were still hanging on the stem a month after inoculation and only 1 remained on the tree longer than normal.

To investigate if this premature dropping of the leaves will prevent the entrance of the fungus through the petiole into the stem, cultures were made of 34 petioles as soon as possible after the leaves dropped. From 4 petioles *Phytophthora syringae* was isolated but only from some parts of the petiole, never from all. Still this indicates that with strong development of the fungus under favorable conditions the fungus may be able to enter from the petiole into the stem. The following observation strengthens this opinion. The inoculated leaves were a source of new infections on the neighboring ones, the lower leaves especially being infected by the falling drops loaded with fungus spores which developed abundantly under the moist cotton wool. The result was that after some time not only the inoculated leaves but the majority of them, belonging to the trees used, were affected. It was on 5 branches of these plants that for the first time natural infection of the cortex and buds was observed in winter in the experimental plot. This indicates even where the special inoculations of leaves had no direct results, to look to the affected leaves as the natural infection source of the branches in nature.

A search was made in the autumn of 1923 for diseased leaves with brown petioles. Only a very few were discovered, in Aalsmeer as well as on the experimental plot, which remained on the stem for an abnormally long period. Their leaf traces differed from the normal ones through a dark violet color, while all the surrounding tissues were quite healthy (Fig. 4). In this dark part mycelium was found. Isolations of the fungus were tried; they had no result in November, but in December from 7 of 8 colored leaf traces *Phytophthora syringae* was isolated, while in 10 normal green ones from the same branches no fungus was found. Two branches of this last plant grown under similar conditions were left on the stem and they were the only ones to show natural stem infection in 1924. It is thus proved that under special circumstances diseased leaves can be the cause of the attack on the stem.

Anatomy

Mycelium of *Phytophthora syringae* was present in the cortex between the green cells at a distance of 4 mm. from the brown part. The brown color

is thus not due to an action of the fungus in advance. The disease spreads along the cambium, then the inner layer of the cortex between the sclereid cells and cambium is attacked and then the outer layer. The distance, however, from the place where only the cambium is affected to that where the whole cortex is brown, is on the average less than 1 cm.

The presence of the mycelium is not restricted to the cortex but it was also discovered in the wood-vessels. In the wood it is mostly found near the cambium, sometimes extending further, once it was even present in the pith. The wood acquires a dark violet color, as has already been described for the leaf trace. To prove that it is really *Phytophthora syringae* and no secondary fungi which are present, cultures were made of the wood after removing very carefully all parts of the cortex and Phytophthora was isolated.

DISCUSSION

Klebahn (5) has already shown that stem inoculations are only possible after wounding and as the fungus can only live in the cortex during winter, it is not very probable that in nature stem infection takes place. An isolated case may be the result of wound infection, but it can never be the cause of an epidemic.

It is more likely that buds are the place of entrance of the fungus, still this disagrees with the facts known. In Aalsmeer in October, 1922, many affected leaves were found, proving that the fungus was generally present. November and December were very moist and the experiments showed that during these two months the buds are most susceptible, still not a single diseased branch was observed during the whole winter. If the buds are the place of infection, it is likely that the disease would be found more regularly, as buds are susceptible during the whole winter and on the average some moist period occurs during that time.

As neither the stem nor the buds seem to be the place of infection, the affected leaves may still be the cause of the attack on the branch. As has been shown previously the mycelium may enter from the petiole into the stem in certain cases. At the moment of leaf dropping the fungus is not yet able to live in the cortex, but it stays in the leaf trace, its presence there being easily detected by the violet color of the tissues. The fungus keeps alive in the leaf trace till the condition of the cortex enables it to live from the cortex cells, which happens in December. From here it spreads attacking the buds from inside. If this really is the natural mode of infection, the buds will not be killed until January. This corresponds with the known facts. In 1913 in Aalsmeer the diseased buds were not noticed till after January 1 and it was observed that the inner bud scales were often the first to acquire a brown color. If a plant harboring the fungus in its

leaf traces is used for forcing before January not a trace whatever of the disease will be found, as the experiments showed that the development of the fungus stops as soon as the tissues of the host plant starts growing.

Also the occurrence of epidemics is easily explained by the leaves being the place of entrance of the fungus. The affected leaves have a tendency to drop prematurely, thus in normal cases no attack on the stem will take place, which corresponds with the entire absence of the disease in most years. It will only be under abnormal circumstances that the fungus will be able to enter into the stem from the petiole before the leaves drop. Investigations of the weather charts to see what conditions can have been the cause of special circumstances in 1912 and 1918, showed that in both years an abnormal rainfall was recorded in August and September. In 1912 precipitation occurred on 37 days between August 1 and September 10, while in 1918, of the 30 days of September 29 were rainy. This amount of moisture will probably enable the fungus to develop on the leaves to such an extent that the mycelium can enter the stem before leaf dropping. Besides it will also be favorable for the dissemination of the fungus as sporangia will be formed in great quantity on the diseased leaves.

It has been shown previously (3) that *Phytophthora syringae* can easily grow in the soil as a saprophyte. This accounts for the fungus being generally present throughout the year. If abnormal rainfall occurs in some period other than August or September, it will also cause strong development of the fungus in the soil and consequently on the leaves, but it will never result in attack on the stem for the fungus will not keep alive in the cortex as the inoculation experiments have proved.

Thus it is only under very special conditions, *viz.*, abnormal rainfall in August or September, that *Phytophthora syringae* is becoming of economic importance. In general the fungus will be quite harmless, causing only the spotting and dropping of some leaves and if circumstances are not favorable it will not become parasitic, but live only in saprophytic form. Thus it may be counted as one of the hemi-saprophytes. Its parasitism is neither very specialized as is demonstrated by the fact that lilacs and apples both are attacked. Also Klebahn was able to infect other plants than lilacs. This scanty specialization of parasitism is a characteristic of many other species of Phytophthora, the majority of them attacking several host plants, belonging to quite different families. In this genus therefore care should be taken in using inoculation experiments to identify species.

CONTROL OF THE DISEASE

Measures for controlling the disease are only wanted in years when very abnormal rainfall occurs in August or September. The presence of a large number of affected leaves will indicate the danger of a future epidemic.

Even in those years control is only wanted for those plants which are to be forced after January.

The only way to prevent the attack on the stem will be by removing the leaves before the fungus has entered the leaf trace through the petiole. The general method, used by the cultivators to produce leaf dropping, which is necessary for early forcing, by putting the plants in dark under cover, just favors the development of the disease. So the only way to remove the leaves will be through picking by hand.

Bordeaux mixture also causes the leaves to drop but it may injure the buds to some extent at the same time, thus diminishing the value of the flowers. Experiments are still in progress with lime-sulphur, but no definite results are yet obtained.

SUMMARY

Phytophthora syringae not only attacks the buds and stem of lilacs but it also affects the leaves.

Conditions present in growing tissues prevent the development of the fungus, thus it can only live in the cortex of the lilac from December till April, while buds are only susceptible from October till February. In nature, bud infection does not seem to take place.

In general, affected leaves drop prematurely, under special circumstances; however, the fungus is able to enter through the petiole into the stem. The natural attack on the stem and buds probably occurs in this way. The special conditions needed are abnormally plentiful rainfall in August or September.

Control measures are only wanted in those years and only for lilacs to be forced after December. Picking of the leaves by hand is recommended.

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STANDARDIZING OF DEGENERATION DISEASES OF POTATO

H. M. QUANJER

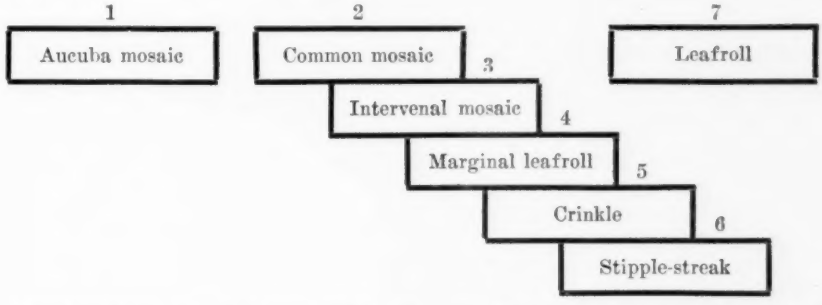
One of the first needs in the study of the potato-diseases, which in former years were thrown together as "curl" or were regarded as symptoms of degeneration, is the means of identifying each of them. Descriptions have been published for this purpose, illustrated by drawings (3) and water-colors (2), which give a clearer idea of their character than photographs. A collection of standard types is planted every year on the grounds of the writer's laboratory at Wageningen. The method of raising these "pure cultures" is the same as has been demonstrated during the International Conference for Phytopathology and Economic Entomology, Holland, 1923, by Dr. Oortwijn Botjes on the potato-selection farm at Oostwold-Oldambt for the raising of healthy strains of numerous potato-varieties. The Board of Agriculture has chosen this farm for this purpose, since no potatoes are grown in its vicinity and since the climate at Oostwold, on account of its situation near the North Sea, is not favorable for the spreading of the "degeneration diseases." The method is based on three principles: Selection, lifting in immature condition, and isolation. This selection farm is an example for improvement of potato culture in the whole country and at the same time supplies the disease-free plants for the laboratory at Wageningen (1).

The pure cultures of degeneration diseases are grown on the grounds near the writer's laboratory, situated amidst meadows at great distance from other potato-fields and exposed to the west-winds, a situation which is less favorable to the spread of these diseases than a sheltered garden would be. Seven diseases of this group have been demonstrated in pure form, during the Conference; there are more than seven, but in not all of them has the isolation in pure culture succeeded thus far.

A great difficulty in this work is that of establishing whether a certain pathologic condition of some potato variety, with which one is not familiar, is new or due to the one or other of these diseases.

It is a well-known fact that the symptoms of these maladies vary somewhat on the different varieties. Some of them are more heavily attacked and seem to degenerate more rapidly than others. In some the yield decreases markedly to remain then at a minimum for a number of years; in others failure to form any tubers and dying out of the line takes place in a couple of years; in still others the decrease in yield is so small, that it is difficult to say whether it is due to the disease or to environmental conditions.

It would be wrong, however, to suppose that these differences are so great and so marked that what may be, for instance, mosaic in one variety is stipple-streak in another or that the various maladies of this group are one and the same disease on various varieties or even one and the same disease under different environmental conditions. When potato plants of the same varieties are infected with the various known virus diseases and kept under the same environmental conditions they will produce complexes of symptoms that are decidedly distinct for each of the diseases and this distinction is constant, so long as the environmental conditions (temperature, moisture, and light) remain constant. The varietal difference of symptoms under consideration here is of another nature. All varieties infected with crinkle, for instance, show more or less typical crinkle symptoms, there are varieties that might display these symptoms in a much more pronounced form so that some of them may begin to resemble some of the symptoms of stipple-streak. On the other hand, there are varieties which, when infected with crinkle, may show the symptoms of the disease in such a slight form that some of them begin to resemble some of the symptoms of mosaic. It is therefore an overlapping of symptoms and not a changing of symptoms. This overlapping of symptoms is shown graphically below as it actually takes place.



Overlapping takes place only between Nos. 2–6. The intervarietal infections are useful not only in studying the relative severity of the various diseases on the various varieties but also for identifying pathologic conditions of unknown varieties. The diagnosing of a given potato virus disease with certainty is possible in most cases, only when we transfer it to one or more potato varieties whose behavior towards all of these diseases is well known. For this reason the "pure cultures" are kept growing in Wageningen in some of the Dutch standard varieties.

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METHODS OF STUDYING THE DEGENERATION DISEASES OF POTATO

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INTRODUCTION

The work on the well-known problem of degeneration in the potato, *Solanum tuberosum* L. has made valuable contributions during the last ten years towards the better understanding and solution of this problem. Unfortunately the complexity of the problem increases more and more with every new contribution towards its better understanding.

The problem was comparatively easy when there was known but one degeneration disease of this plant or rather when all degeneration diseases were known as one disease under the name of curl (2). It became two times or actually three times as complicated when the original disease was segregated into mosaic and leaf roll, and by the time the original disease was segregated into seven or eight different degeneration diseases the difficulties of the plant pathologist became still greater. The probable interrelation of some or all of these diseases to similar diseases of other plants and the actual existence of masked carriers of these diseases made the difficulties still greater.

All of the above named difficulties lay in the nature of things and while, in most cases, nothing can be done to lessen them, everything should be done to avoid increasing them. This is what the great majority of workers on these diseases have apparently neglected to do: First of all they have failed to give us in most cases exact and full descriptions of the symptoms and development of the various diseases during their various stages as seen in a certain variety and under given environmental conditions. Most descriptions of such diseases are so short and incomplete that no one can ever recognize the disease by using the existing descriptions, while the great majority of the illustrations of such diseases are of no diagnostic value whatever.

Most of the existing descriptions of such diseases are full of terms and expressions that have no distinct value for the reader. Unquestionably the authors of such terms associate certain diagnostic values with words like "dwarfing," "wrinkling," "streaking," "browning," "curly dwarf," "rusty dwarf," "yellow dwarf," "mosaic dwarf," "rugosity," etc., but for the reader such words are meaningless because they are too broad and too general and because they are not sufficient in themselves to describe a whole complex of symptoms. What is still worse is that one author calls a certain disease "leaf roll," another author speaks of the same disease as

"leaf curl;" one uses the name "streak," the other the name "stipple-streak" for the same disease; one speaks of "crinkle," the other of "rugose mosaic" and they both mean the same disease; one calls a certain disease "common mosaic," a second calls it merely "mosaic," while a third uses the name "mild mosaic" or "medium plus mosaic" for the same disease; one speaks of primary and secondary form, the other of current season or first and second season symptoms.

The only result of this deplorable practice is that even the men working on this problem cannot understand each other's writings, let alone those who are not familair with these diseases.

It is really time for the various workers on this problem to come together to put an end to this chaotic and deplorable condition (15, p. 127, 128).

The aim of this paper, while offering no fundamentally new facts and opening no principally new perspectives, will be to outline some methods and offer some suggestions which may help to simplify and speed up the work on the virus diseases of the potato plant.

PRELIMINARY FIELD STUDIES AND COLLECTING OF MATERIAL

The first thing in the studying of any or all of the degeneration diseases of the potato plant is to learn what a healthy, normal, and vigorous potato plant of given variety looks like. It is useless trying to study the degeneration diseases of this plant (17) without knowing what a disease-free plant should look like.

The symptoms of most of the potato degeneration diseases vary considerably in the different varieties but they are constant on the same variety and under given climatic conditions. As a rule the varietal difference of symptoms consists on the one hand in a more pronounced, on the other hand in a less pronounced expression of the complex of symptoms accompanying each disease. So that the same disease might bring about in the different varieties shades and degrees of a given complex of symptoms. And it is indeed possible that certain of the symptoms, but not all symptoms of a disease, might be entirely absent on some varieties and very pronounced in others. For instance, tubers of stipple-streak plants may show pronounced peridermal discolorations (1, p. 8) in some of the highly susceptible varieties and no discolorations of the tubers whatever in other varieties. As a rule it can be said that the rapidly growing varieties with luxuriant, tender, and light colored foliage show more pronounced symptoms than the hardy, slow growing, and late varieties do. It is therefore advisable for everyone to limit his work, at first at least, to one variety and preferably to a variety which has been already used successfully for such studies by former workers. As such varieties may be mentioned: Green Mountain, Paul Krüger (President), Bliss Triumph, Bravo, etc.

The variety known in Holland under the name "Schotsche Muis" and in England under that of Victory, which is an unusually early potato, rapidly growing, early sprouting and highly susceptible for all kinds of diseases, will prove, in the writer's judgment, to be an excellent variety for studying the degeneration diseases of potato.

In trying to get a general idea of the various types of degeneration diseases of the potato plant, it is absolutely necessary to limit one's studies and observations exclusively to fields that are practically free from any degenerated plants. The better and the more luxuriant the condition of the plants and of the field in general, the greater is the chance that a portion of the few diseased plants will be in the primary stage of the disease. The newly infected plants in a first class field that have just got any of these diseases are much more apparent and the recognition of the diseased plants and the diagnosing of the disease is much easier than in a field where most of the plants are infected or otherwise injured. The slighter the symptoms of the diseased plants are the more suited they are for further study, since in most cases their disease will prove to be pure, *i.e.*, only one disease being present in each infected plant. Such plants when standing far from any other diseased plants should be marked and watched for the further development of the disease and eventually for the collecting of material. Such plants have the size, general appearance, and vigor of the healthy plants and cannot be distinguished from a distance. They can be recognized only upon a close and careful examination. The symptoms of the degeneration diseases become apparent first in the young and rapidly growing tops of one of the shoots; from the tops they advance downwards in the shoot and then in the sister shoots of the same hill.

Material, unless otherwise desired, should be collected or the plants dug out not before the symptoms have become distinctly visible in all shoots of a hill, but as soon as possible afterwards. If we get material or dig the plants at an earlier date as indicated above we are apt to collect material and tubers which have not yet been reached by the virus, on the other hand if one should delay too long digging the plants or collecting living material, for instance for sap or for graft infections, the plants may be meanwhile infected by some of the other virus diseases. The harvested tubers should be washed carefully without injuring their periderm, dried, fumigated,[1] to make sure that they are free from any insects, properly labeled, and stored. The washing is necessary because it enables us to see the effects of the disease on the tubers if such should be present, as they actually become apparent on some varieties (1, p. 12).

[1] Exposing the tubers for several hours to the fumes of nicotine extract (250 c.c. for every 40 m^3 of space) will kill most insects that may be present on the tubers.

It is in most cases not advisable to collect material from plants that are in an advanced stage of the disease, first because most of the degeneration diseases in their advanced stages exhibit symptoms which are common to most of them, and which are much less typical than the respective symptoms in their primary form, and secondly because such plants as have been exposed to contamination for many generations may carry more than one disease.

There are at present no reliable methods for distinguishing, separating, and getting in pure condition the two or more diseases that may be present in the same plant, so that it is perfectly useless to experiment with such plants or tubers, which represent an unknown quantity. Those desiring to study combinations of degeneration diseases should self-infect healthy plants simultaneously with two or more of such diseases, instead of trying to analyze existing combinations of diseases.

Fields to be used for the studying and selecting of virus diseases should never be sprayed, because this masks many of the disease-symptoms. Such fields should be as free from insect injuries as possible, especially from flea beetles (*Epitrix sp.*), Colorado beetles (*Leptinotarsa decemlineata Say*), aphids, etc.

INFECTION EXPERIMENTS

By far the greatest difficulties in this problem (outside of absence of visible pathogenes), lies in the somewhat longer incubation period of these diseases, under ordinary conditions and in the infection work. All this makes it necessary first to protect the inoculated plants from possible external contamination by insects or possibly other agencies, which is very expensive, in some cases impossible and requires much work and time. In the second place, should the symptoms of the disease fail to appear during the first season the tubers of every inoculated plant must be planted the following year. This means an increase in work and expenses equal to from five to ten times those of the first season, since for every inoculated plant of the first year we shall have to attend to from five to ten plants during the second year. Should one begin with several hundred of plants in the first year, the following year their number will amount to several thousands and the further the work proceeds the greater this number will become, so that before long the work must naturally become less productive, the chance of error increases to far beyond the permissible, while the financing of the problem become an impossibility. On the other hand, the very length of the experiments make their eventual results very doubtful, and not seldom the results may be not only negative but directly opposite to what they should be. It often happens that the plants infected with mosaic will develop leafroll in the second generation while their checks may show (for instance) mosaic symptoms. Schulz and Folsom (19, p. 65)

write also that "sometimes observations on the first generation in an experiment including the controls, are more valuable than those on the second generation." When a certain experiment fails to give final results during the first season it is far better to repeat it the following year than to try to get results by growing further the progenies of the infected plant.

All of the above difficulties would disappear automatically if it should be possible to modify the present methods of experimenting on these diseases in such a way that in all cases the infection-experiments could be completed during the same growing-period. This is not only possible but it can be accomplished with great ease and requires negligible expenditure, while the chance of error is practically diminished to zero and the probability of success becomes much greater.

Before discussing the methods by which the above can be accomplished, it may not be out of place to state that the old method of two-seasons-infection-experiments has made possible the important discoveries concerning the nature of the degeneration diseases, which form the foundation of our present knowledge of said diseases. This method was originally worked out in Holland by Quanjer and Oortwijn Botjes, afterwards it has been used by Murphy and Wortley, by Schulz and Folsom, and the writer.

Sprout Infection

That some of the diseases under consideration can be transmitted to sprouted tubers with certainty and great ease in the shortest possible time and with 100 per cent of positive results has been experimentally established by Murphy (13, p. 178), Schulz and Folsom (19, p. 87 and 91), and by the writer (3). There is no reason why the rest of these diseases could not be transmitted in same way. When infecting potato plants by means of sap, grafting or aphids, we can never be absolutely sure that the plant we are to infect is not already infected with the same or another disease, since absence of symptoms is in no case a guarantee that the plant is disease-free and the control plants can never serve as such, because they as well as the inoculated plants might get infected at any moment during their comparatively long vegetative period, as has been already pointed out. This is especially evident from Schulz and Folsom's last work on this subject (19) where in some cases the infected plants remained healthy while the checks became infected.

In most cases it is not possible to cage the inoculated and control plants so as to make absolutely sure that no infection from outside has taken place. This however can easily be accomplished if one works with sprouted tubers as it is possible to handle a tuber in such a way that it will never come into contact with insects. Moreover portions of the same tuber may serve as controls by forcing them beforehand, thus making it possible to ascertain

in advance whether the tubers to be used for the infection-experiments are disease-free or not, which can never be done with equal certainty when plants are used.

Schulz and Folsom (19, p. 51), experimenting with flea beetles as probable mosaic carriers, had difficulties in keeping the experimental plants free from aphids; they write: "In spite of special precautions, potato aphids were introduced with the flea beetles into some cages." "The difficulty with aphids is chiefly one of supplying the proper control conditions for inoculations on a large number of plants in the field" (19, p. 48). The certain exclusion or caging of aphids is a difficulty experienced by many workers.

Still another disadvantage of plant infections is that when aphids are used as disease carriers they may produce on the plants distinct mottling, crinkling, and chlorosis even when non-virulent, thus making impossible the interpretations of the presence or absence of certain symptoms. Besides this it necessitates the spraying or fumigating of the plants in order to free them from the insects if this becomes necessary.

A. *By aphids*

The most rational method of infecting sprouted tubers is by means of aphids. This has been done with potato-stipple-streak (3), mosaic (19), and leafroll (13); it can be done as follows:

The first thing needed is to secure a quantity of disease free aphids. In a very limited number of cases the writer obtained what proved to be disease-free aphids in the following way:

A number of full-grown aphids taken from diseased or healthy plants were placed on the sprouts of a healthy tuber which was then placed in a glass jar. To prevent other insects from entering the jars it is necessary to tie up a cloth around the opening of the jars. The cloth should be so fine that even the smallest aphids should not be able to pass through it. Many workers use for such purposes ordinary cheesecloth, this is however far from being adequate. The writer has been using a much finer fabric having 20 meshes per one centimeter in both directions, the space between the meshes being 0.35 mm. and he has repeatedly observed that the smaller aphids did pass freely through such cloth and left the jars even when there were no other tubers or plants in the neighborhood to attract them. The space between the meshes should not be more than 0.2 mm.

If the temperature of the room is not less than 18° C. new aphids are born at a quite rapid rate and when transferred immediately after their birth by means of a fine hair brush to the sprouts of a second healthy tuber, they proved to be free from any virus disease. Within two weeks the young aphids reach their full development and begin to propagate at an

astonishing rate, so that a few jars with sprouted tubers are sufficient to supply any quantity of aphids. The jars with aphids should be placed in the light. New jars with fresh tubers should be set every second or third month. Whether this method will give disease-free aphids in all cases and under all conditions is far from certain. The recording of this fact is worth the while in connection with McClintock and Smith's work on spinach mosaic, who showed that in the case of this disease even the 3d and 4th generation aphids were infectious.

The aphids so obtained are then placed on the sprouts of infected tubers. So long as the minimum period needed for the aphids to become infectious after feeding on an infected plant or sprout is not known, the aphids should not be removed from the infected sprouts and used for infection experiments before they have spent ten days on the infected tubers or plants. After securing a reserve of virulent aphids a number of full-grown aphids (10–15) are transferred to the one half of one or more sprouted healthy tubers, which have been cut longitudinally in two pieces and placed in a glass jar, while the second half or halves, are placed in a second glass jar.

Both jars, with and without aphids, are then placed for several weeks (6–8) in a room at a temperature of 20° C. to incubate. The room should be moderately moist and light. In this way it is possible to bring a maximum of inoculation into a minimum of plant cells, while the tender and growing sprout offers ideal conditions for the multiplication of the virus. Should the jars have been kept in darkness, which is not desirable, they must be placed before planting in the sun light for several (10–15) days. This is necessary for the hardening and thickening of the sprouts, otherwise they are apt to fall off during the planting. In the case of stipple-streak and in some cases of crinkle the symptoms of the disease may become apparent on the tubers and the sprouts within 5–6 weeks. But an incubation period of two months is to be preferred in most cases. The incubation of the tubers, as indicated above, is necessary for intensifying the infection. Tubers thus treated are fumigated to free them from the aphids and together with their controls are then planted at a time and under conditions that are known to be favorable for the development of the respective disease.[2] Care should be taken not to break off the sprouts. Such tubers, if only the soil temperature and moisture are favorable, will send shoots above the ground within a very short time and usually much earlier than tubers

[2] Before we learn something more regarding the optimum temperatures of the various virus diseases it will be advisable to keep the pots with the infected tubers at 18–20° C. A medium moisture of the air and a proper moisture of the soil, that will facilitate the rapid development of the plants, are also essential. In the absence of sufficient sunlight the plants grow very abnormally, which in turn precludes the appearance of typical symptoms.

planted without sprouts. The check tubers will form normal plants, because even if they should have been infected after their planting in the field the time during which they have been exposed to infection has been too short for the development of any symptoms. The plants from the infected tubers on the other hand will show in most cases pronounced symptoms of the secondary form of the disease, if only the temperature has not been too low. Herewith the particular experiment is definitely finished, within a minimum length of time, with no special expenses and work for caging of the plants and with a minimal chance of error or complications.

Should it be desired to obtain plants also with the primary form of the disease in the case of stipple-streak or gradations between primary and secondary symptoms of the respective disease one needs only to lessen more or less the period of incubation or to plant the incubated tubers in cooler soil.

An even better method of accomplishing this is by grafting a portion of a diseased tuber to the freshly cut half of a healthy tuber, tie them firmly together, let them stay for two days at room temperature, then plant them in pots. At first the shoots coming from the diseased half will be also diseased, those of the healthy tuber will remain healthy for some time and then show suddenly in their tops symptoms of primary infection.

B. *Sprout grafting*

Sprout grafting is not only much easier but it possesses all the advantages of the sprout infections and lacks all of the disadvantages of the infection of plants.

Sprout grafting is possible even in cases where plant grafting is impossible as in the case of stipple-streak: Most potato varieties when infected with stipple-streak wilt and die so soon that a grafted scion dies before the infection has passed into the grafted plant (1, p. 20). For sprout-graft infections it is necessary to place the infected tubers to sprout at a temperature of about 20° C. If lower or higher temperature are used the probability is not excluded that under certain conditions there may be formed sprouts that are free from the disease during a given period of time and may remain so if prematurely detached from the mother tuber. Whether this actually can take place is not positively known, but some field observations have suggested to the writer this possibility. The tubers of which sprouts have been grafted are placed for several days (10–15) in a moist chamber to facilitate the growing together of the graft and scions, they are then incubated at 20° C. for 5–6 weeks as described under sprout infection. Care should be taken that no insects come in contact with the tubers. The tubers should be kept in the light if possible for reasons already mentioned.

C. *Grafting of tubers*

Tuber-grafting, first used by Quanjer (15), is as useful as sprout grafting, only that in this case much more infected material is needed. In the first case several infected tubers can supply us with a large number of sprouts during a period of several months, whereas in the second case one infected tuber can serve for not more than 2 or 3 tuber graftings. The grafted tubers well protected from insects are incubated as usual in light rooms at 20° C. for 6–8 weeks, then planted if environmental conditions in the field are favorable.

Plant Infection

The results of plant graft infections are as instructive and as evident as the sprout infections are striking and should be used whenever possible, both as a check to other infection methods and as the only reliable method of infection where other methods may not be possible or are difficult as in late summer when tubers are no more available.

By far the largest part of the infection work done with the degeneration diseases of potatoes has been done with plants. Hence the greater popularity of this method. Here need only be emphasized the following two facts: The plants to be used for aphid, graft, or sap infections must be young, vigorous, and rapidly growing. Plants that are stunted, weak, of advanced age, slowly growing, or injured should never be used for infection experiments as very often they fail to show any symptoms.

Of just as great importance are the environmental conditions under which the infected plants grow. The temperature should not be much below or above 20°, the soil not dry and the air humidity not too low. The infected plants should get as much sunlight as is possible, since only a normal development of the foliage will make possible the appearance and recognition of the symptoms. The infections should be made at such a period of the year that the plants can pass at least two full months in active and normal growing before the appearance of any symptoms of ripeness.

Sap infections give better results when made on the leaves, preferably by leaf mutilation as used by Schulz and Folsom (19).

A. *Plant grafting*

When done early in the season with young and rapidly growing plants and under favorable environmental conditions the plant graftings also give final results during the same growing period. Next to the sprout infection method the plant grafting makes possible the transmission of a maximum of inoculum into the grafted plant.

This method is especially adapted for interspecific transmission of virus diseases. The studying of interspecific relationships of the virus diseases

is of great scientific as well as of practical importance. Such studies will also throw light upon the problem of alternate hosts which might serve to perpetuate and spread these diseases. This method of infection, first described in 1916 (15), makes it possible to watch and follow up the advancement of the disease from the scion, first into the nearest standing growing point, then into the next standing branches of the same shoot and finally into the sister shoots.

The freshly grafted plants must be kept for several days (6–7) in moist chambers, well supplied with light, to facilitate the growing together of the scion and the graft.

B. *Aphids*

The successful graft infections should be repeated with insects, since the possibility is not excluded that by the graft-method it may be possible to transmit in some cases a particular disease to a plant to which it is never transmitted in nature and even to produce distinct pathologic changes, in some cases resembling those of the natural host. In this connection it must be emphasized that none of the above methods of infection alone will be sufficient to establish the identity or difference of given virus diseases of different plants. Further investigations and finer methods, peculiar for each case, will be needed for settling such questions.

Failure to produce any symptoms on the grafted plant is no proof that the virus present in the scion has not passed and spread throughout the same, since there are many known cases of the so-called "susceptible masking" hosts or symptomless carriers (15, p. 132). The possibility of the existence of intermediate hosts (7, 19, p. 88) should also be borne in mind. It may be possible to transmit a certain virus disease to a certain plant only by transmitting it first through an intermediate host.

For infections with aphids one should take several tuber-sprouts covered with aphids from the aphid stock-jar and place them on the tops of the caged healthy plant. If one transfers only the aphids one is apt to injure a larger percentage of the aphids. Less reliable for this purpose is the method used by Schulz and Folsom (19), which they call aphid dispersal, *i.e.*, one healthy plant is caged with a diseased plant on which aphids are present. In this case the rôle of soil and other insects, which may possibly circulate under this condition from plant to plant, is not excluded. So long as this subject has not been investigated thoroughly we have no right to suppose that the soil insects play a rôle in the transmission of such diseases. And indeed the writer has some circumstancial evidence that wire worms may play a part in the spreading of stipple-streak and possibly of other diseases.

INCREASING OF THE INFECTED MATERIAL

Not seldom the infected material (tubers) of a certain disease or of a particular variety actually suited for further experimenting may be so limited that no work on any scale can be undertaken with such material. Under the present conditions such material has to be planted the following season with the hope of obtaining more tubers which is not always done; besides it takes a great deal of time. This can be done with a much greater certainty and in much shorter time by grafting portions of the infected tubers to large and healthy tubers of the desired variety. The piece of the infected tuber is tied firmly against a freshly cut surface of a large and healthy tuber. The grafted tubers are kept at a temperature of 20° C. for 6–8 weeks to incubate them thoroughly. After the incubation the tubers are thoroughly infected and can be used further as infection material. In this way it is possible to increase the material several times, since every one of the small diseased tubers will give 2–3 large tubers after the grafting. If desired, the newly infected tubers can be used further for the multiplication of the diseased stock. The writer has used this method with the stipple-streak disease with great success.

TIME OF FIELD PLANTINGS

The potato can grow and develop a normal and very luxuriant growth at comparatively low temperature if only sufficient sunlight is present. Most of the virus diseases of this plant on the other hand require a much higher temperature for their development so that their development can not only be retarded but even completely suppressed by low temperatures which are high enough to permit a normal development of the plants. Under such conditions one may obtain apparently normal plants from positively infected tubers (1, p. 22), which continue to grow normally until after the coming of warmer weather. Meanwhile the plants reach their full development, and as such usually fail to show any symptoms of the disease even after the rising of the temperature. The young leaves, on the other hand, show, after the rising of the temperature, no longer the secondary symptoms of the disease in question, as they should have done had the temperature been favorable for the disease, but the primary symptoms of same; thus giving us an entirely different result from what it would have been had the plants been grown from the very beginning at a temperature favorable also for the development of the disease. It is nothing unusual for such plants to form a large number of fully developed tubers, whereas at temperatures favorable also for the development of the disease, they may not form any tubers at all.

Field plantings, therefore, should never be done at a time when the soil and air temperature are too low, that is, field plantings of infected material

should not be done too early in spring, and yet as early as possible, because a too late planting, as has been pointed in an earlier chapter, will increase the chance of outside contaminations and might thus decrease the value of the results.

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PHYTOPATHOLOGICAL NOTES

Institutions for Phytopathology and Economic Entomology in Holland.—Until recently the Dutch Phytopathological Service was connected with the "Institut voor Phytopathologie" under the directorship of Prof. Ritzema Bos. The scientific work of the "Instituut" was by this arrangement seriously hindered. After the retiring of Prof. Ritzema Bos, the service has got its own Director, Ir. N. van Poeteren, with headquarters at Wageningen, its task is inspection, advisory and extension-work. The task of the "Instituut voor Phytopathology," which is a department of the University of Agriculture, Wageningen, is teaching and research. It has now three laboratories: in the original building the laboratory for entomology is now being equipped under the direction of Prof. W. Roepke. A new building at Wageningen has been built for mycology and potato-research under the direction of Prof. H. M. Quanjer. The third laboratory, also newly built and equipped for flower-bulb research, situated at Lisse, amidst flower blub fields, is directed by Dr. van Slogteren.

In the second of these laboratories the teaching is chiefly done by Prof. Quanjer, assisted by Miss J. H. H. v. d. Meer; research is the main work of Miss H. L. G. de Bruyn, Dr. D. Atanasoff, and Ir. S. J. Wellensiek, and a number of graduate students. About 15 acres of ground belongs to this laboratory; besides the subsidized potato-selection farm of 120 acres, under the direction of Dr. Oortwijn Botjes, at Oostwold, Oldambt, the aim of which is to help the laboratory in growing healthy potato varieties for infection experiments (*c.p.*, this No. of Phytopathology: "Standardizing of degeneration diseases of potato)."

The Phytopathological Laboratory, "Willie Commelin Scholten," the seat of the "Central Bureau voor Schimmelcultures," is an endowed institution for phytopathological research. It is directed by Prof. Johanna Westerdijk, who also teaches phytopathology at the department of botany of the University of Utrecht.—H. M. Quanjer.

Höstermann, Gustav, Noack, Martin. Lehrbuch der pilzparasitären Pflanzenkrankheiten mit besonderer Berücksichtigung der Krankheiten gärtnerischer Kulturgewächse, 271 pages, 104 text figures, 1903, Paul Parey, Berlin. Price M. 9. This volume is intended to fill the gap in the more general works of Frank, Sorauer and V. Tubeuf and is devoted more especially to the diseases of garden crops. The introductory matter on the classification of the important groups of fungi concerned in disease is well written and helps the student to obtain certain necessary fundamentals for the special sections which follow. The practical considerations in the treat-

ment of the important pathogenic groups are clearly presented. A special and helpful feature of the book is a key to the more important fungi which cause disease in garden crops. The key is arranged according to the host plant and will be most helpful to the beginner. The illustrations are selected with considerable care to present the subject in hand. The volume should prove useful to all students of plant disease.—JAMES R. WEIR.

Endothia parasitica. Dans une communication faite à la sernière réunion de Cincinnati de l' "American Phytopathological Society" et reproduite dans le numéro de janvier dernier de ce journal (vol. 14. No. 1, p. 52) M. Haven Metcalf a annoncé la découverte qu'il avait faite, en Belgique, à Bruges, du redoutable *Endothia parasitica* du Châtaignier.

M. Marchal, Directeur de la Station de Phytopathologie à Gembloux me fait savoir, à ce sujet, qu'il s'est livré, avec la collaboration de M. Van Hove, chef du Service d'Inspection phytopathologique, à une enquête, à Bruges et dans les environs.

Il résulte de leurs recherches que le cas observé par leur savant collègue américain devait être un cas tout-à-fait isolé car toute trace d'Endothia a actuellement disparu.

Ils concluent de leur enquête que l'on peut encore considérer, à l'heure actuelle, la Belgique et l'Europe comme indemnes de ce champignon.—H. M. Quanjer.

Third Pan-American Scientific Congress Postponed. Notice has been received that the Third Pan-American Scientific Congress which was scheduled to commence November 16, 1924, has now been postponed to December 20. The reason given for this postponement is that more scientists will be able to attend during the latter part of December than during November. The period during which papers or summaries thereof, which do not exceed 1,500 words, will be received has been extended to November 1.

Annual Meeting of the American Phytopathological Society.—The Society will hold its meetings at the Central High School, Washington, D. C., during the three day period December 30 to January 1. As usual this meeting is in conjunction with that of the American Association for the Advancement of Science. In addition to the customary joint session with Section G the Society will meet with the Mycological Section of the Botanical Society of America for the presentation of papers of common interest. At least one session will be devoted to papers on pathological subjects which are in the nature of finished pieces of research. The majority of the papers, however, will probably be presented to two or more groups of the Society

in simultaneous session. An attractive additional feature of the meetings this year will be the opportunity to visit the Department of Agriculture and other points of interest about Washington. The Phytopathologists' Dinner with its entertainment program will take place on Thursday evening, January 1.

The New Ebbitt Hotel at 14th and F Streets will be the headquarters for the Society and for the Botanical Society of America.

Laboratory facilities at Naples, Italy. Any biologists wishing to do laboratory work in Italy during 1925 should consider the facilities offered by the Stazione Zoologica at Naples. Because of a grant made to this station by the American Association for the Advancement of Science the association is entitled to name the occupants for a "table" at that station, where, according to the Director, Dr. Dohrn, arrangements have been made for work with plants as well as animals.

Any American or Canadian biological worker is eligible and if there are any interested they should communicate with the permanent secretary of the American Association, Dr. B. E. Livingston, Smithsonian Institution Building, Washington, D. C.

Personals. Dr. Caroline Rumbold, formerly in the office of Sugar Plant Investigations, Washington, D. C., is now in the office of Forest Pathology, Madison, Branch, Old Soils Building, Madison, Wisconsin.

Dr. F. L. Stevens will attend the Pan-American Scientific Congress in Peru as a delegate from the University of Illinois, from the American Association for Advancement of Science, and from the American Phytopathological Society.

Dr. L. W. Durrell, formerly assistant plant pathologist at the Iowa Agricultural Experiment Station, has been appointed head of the department of botany of the Colorado State Agricultural College.